TOLERO PHARMACEUTICALS
Sumitomo Dainippon Pharma Global Oncology

AMENDED CLINICAL STUDY PROTOCOL

Study Title:	A Phase 1, Open-label, Dose-escalation, Safety and Biomarker Prediction Study of Alvocidib and Cytarabine/Daunorubicin (7+3) in Patients with Newly Diagnosed Acute Myeloid Leukemia (AML)
Protocol Number:	TPI-ALV-101 Amendment ***8***
IND Number:	057729
Study Drug/Agents:	Alvocidib (formerly flavopiridol)
Phase of Development:	Phase 1
Medical Monitor:	[redacted]
Sponsor Contact:	[redacted]
Sponsor:	[redacted]
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Date of Amendment 4:	05 June 2018
Date of Amendment 5:	18 April 2019
Date of Amendment 6:	14 June 2019
Date of Amendment 7:	24 February 2020
Date of Amendment 8:	***19 March 2020***

PROTOCOL SIGNATURE PAGE

SPONSOR SIGNATURE

I have carefully read the protocol, TPI-ALV-101 Amendment ***8***, titled "A Phase 1, Open-label, Dose-escalation, Safety and Biomarker Prediction Study of Alvocidib and Cytarabine/Daunorubicin (7+3) in Patients with Newly Diagnosed Acute Myeloid Leukemia (AML)*"* 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-101 Amendment ***8***, 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

5+2	Refers to the regimen of Ara-c: Days 5-9 and Daunorubicin: Days 5 and 6
7+3	Refers to the regimen of Ara-c: Days 5-11 and Daunorubicin: Days 5, 6, 7
ACD	Alvocidib/Ara-c/daunorubicin (alvocidib plus 7+3)
ACM	Alvocidib/Ara-c/mitoxantrone (historically known as “FLAM”)
AE	Adverse event
AGC	Absolute granulocyte count
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
ANC	Absolute neutrophil count
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
CNS	Central nervous system
COV	Close out visit
CR	Complete remission/response
CRA	Clinical research associate
CRi	All CR criteria except for residual neutropenia (<1000/µL) or thrombocytopenia (<100,000/µL)
CRF	Case report form
CR_{MRD-}	CR without MRD
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 (Flavopiridol)/Ara-c/Mitoxantrone (termed “ACM” in this study)
GCP	Good Clinical Practice
GCSF	Granulocyte colony stimulating factor
GM-CSR	Granulocyte-macrophage colony stimulating factor
HCG	Human chorionic gonadotropin
HCT	Hematocrit
Hgb	Hemoglobin

HI	Hematologic improvement
HiDAC	High-dose Ara-c
IC_{50}	Inhibitory concentration in 50% of animals
IEC	Independent Ethics Committee
IND	Investigational New Drug Application
IP	Intraperitoneal
IRB	Institutional Review Board
ITT	Intent to treat (population)
LD_{50}	Lethal dose in 50% of animals
LDH	Lactate dehydrogenase
LVEF	Left ventricular ejection fraction
MedDRA	Medical Dictionary for Regulatory Activities
MDS	Myelodysplastic syndrome
MPFC	Multiparametric flow cytometry
MLFS	Morphologic leukemia-free state
MRD	Minimal residual disease
MTD	Maximum tolerated dose
MUGA	Multigated acquisition (scan)
NCCN	National Comprehensive Cancer Network
NCI	National Cancer Institute
NDNFR	Newly diagnosed Nonfavorable Risk (AML)
NGS	Next generation sequencing
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 1, Open-label, Dose-escalation, Safety and Biomarker Prediction Study of Alvocidib and Cytarabine/Daunorubicin (7+3) in Patients with Newly Diagnosed Acute Myeloid Leukemia (AML)
Study Indication:	Adults with newly diagnosed AML
Clinical Phase:	1
Objectives:	Primary Objective: • To determine the safety and tolerability including the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of alvocidib when administered over a range of doses on Days 1-3 followed by cytarabine/daunorubicin (7+3) on Days 5-11 in adults with newly diagnosed and previously untreated AML. Secondary Objectives: • To observe patients for any evidence of antileukemic activity of alvocidib plus 7+3 using the 2017 ELN response criteria • To establish the Recommended Phase 2 Dose (RP2D) for future studies with alvocidib in combination with 7+3 Exploratory Objective***s***: • To assess levels of minimal residual disease (MRD) using standardized techniques (ie, multiparametric flow cytometry [MPFC] and next generation sequencing [NGS]) and evaluate other potential biomarkers including, but not limited to, MCL-1 dependency
Study Design:	This is an open-label, multicenter, dose-escalation, safety and biomarker prediction study of alvocidib followed by 7+3 therapy in adults with newly diagnosed and previously untreated AML. *Induction* The starting dose of alvocidib will be 20 mg/m^2 as a 30-minute intravenous (IV) bolus followed by 30 mg/m^2 over 4 hours as an IV infusion administered daily on Days 1-3 of Induction. Patients will have a one day drug holiday (Day 4) before initiation of the 7+3 regimen. Beginning on Day 5, cytarabine will be administered as a 100 mg/m^2/day continuous IV infusion for seven consecutive days (Days 5-11) plus daunorubicin administered at a dosage of 60 mg/m^2 IV on Days 5-7. Escalation of the alvocidib dose will follow a 3+3 design with sequential cohorts of 3 patients treated with incrementally higher doses of alvocidib until a DLT is observed and the MTD is established. Three patients within a dose cohort may be enrolled without a waiting period between patients. Escalation to the next higher dose level will not occur until all patients within the current dose cohort have undergone full safety assessments (including count recovery or disease assessment). Dose-limiting toxicities (DLTs) for alvocidib and 7+3 will be determined during **Induction and Reinduction** therapy, determined to be at least

	possibly related to study drug and defined as follows based on the NCI CTCAE version 4.03: • Any Grade 4 nonhematologic toxicity considered at least possibly drug related • Any Grade 3 nonhematologic toxicity considered at least possibly drug related and that does not resolve to ≤Grade 2 within 48 hours, with the following exceptions: o Grade 3 diarrhea, mucositis, nausea or vomiting will be considered dose-limiting only if resolution to ≤Grade 2 (including use of supportive care) requires more than 7 days • Grade 4 cytopenias lasting ≥50 days from the start of the cycle in the absence of residual leukemia if subject received one cycle of induction • Grade 4 cytopenias lasting ≥60 days in the absence of residual leukemia if subject received 2 cycles of induction • Grade 3 or 4 tumor lysis syndrome or related electrolyte disturbances (eg, hyperkalemia, hypophosphatemia, hyperuricemia) that do not resolve to ≤Grade 2 within 14 days • ≥Grade 3 elevations in creatinine that do not resolve to ≤Grade 2 within 7 days • Any AST and ALT elevation ≥5 ×ULN accompanied by serum bilirubin levels >2 ×ULN, regardless of duration • Any Grade 5 toxicity that is not clearly and incontrovertibly related to the underlying disease or extraneous causes • Anorexia, alopecia, fever, neutropenic fever, and infections of any grade should <u>not</u> be designated as DLTs since they are known and expected toxicities associated with the study drugs. If 1 of 3 patients in a cohort experiences a DLT, up to 3 additional patients will be treated at that dose level. If all patients in the expanded cohort have undergone full safety assessments (including count recovery or disease assessment) with no additional DLTs observed**,** the alvocidib dose will be escalated in a new cohort of 3 patients. If 2 or more of 3-6 patients at a given dose level experience a DLT during the first cycle, then the MTD will have been exceeded and up to a total of 6 patients will be treated at the previous lower dose level. If 0 or 1 of 6 patients experiences a DLT at this previous lower dose level, this dose will be declared the MTD. The MTD is defined as the dose at which ≤1 of 6 patients experience a DLT during Cycle 1 with the next higher dose having at least 2 of 3 to 6 patients experiencing a DLT during Cycle 1. Any AEs that occur during Reinduction and meet DLT criteria will be taken into consideration when determining whether to escalate the dose of alvocidib and when defining the MTD. Once the MTD has been established, up to 20 additional patients will be enrolled at the MTD level for confirmation of safety as well as additional

	safety analysis and feasibility for determining MRD by dual methods (ie, MPFC and NGS) at a central laboratory. *Disease Assessment* On Day 14 (±3 days), unless medically contraindicated, peripheral blood and bone marrow samples will be collected for disease assessment including MRD. Patients with **no** evidence of residual leukemia (≤5% bone marrow blasts plus ≤10% cellularity) will be monitored weekly and will undergo response assessment at the time of hematologic recovery (ie, absolute neutrophil count [ANC] >1000/µL and platelet count >100,000/µL) or Day 50 (±3 days), counted from Day 1 of Induction therapy, whichever occurs first. At this time, the patient may continue to **Consolidation** therapy with HiDAC. *Reinduction – Alvocidib and 5+2* Patients **with** evidence of residual leukemia (>5% bone marrow blasts plus >10% cellularity) may be eligible for a second induction cycle (or 'Reinduction**'**) with alvocidib and 5+2 at the discretion of the Investigator. The dose of alvocidib will remain the same, but the 7+3 regimen will be changed to 5+2: cytarabine, 100 mg/m^2/day, as a continuous IV infusion on Days 5-9 plus daunorubicin administered at a dosage of 45 mg/m^2 IV on Days 5 and 6. Should a patient undergo Reinduction therapy, they will be monitored weekly and will undergo response assessment at the time of hematologic recovery (ie, ANC >1000/µL and platelet count >100,000/µL) or Day 60 (±3 days), counted from Day 1 of Induction therapy, whichever occurs first. Upon documented hematologic recovery, the patient may continue to **Consolidation** therapy with HiDAC. Additional bone marrow assessments may be obtained per investigator discretion. Investigators are permitted to administer intrathecal (IT) chemotherapy per institutional protocols after Induction (preferably during marrow aplasia), but before starting Consolidation therapy. Additional doses of IT chemotherapy may be administered after discussion with the Medical Monitor. During both *Induction* and *Reinduction*, if necessary, care must be taken to ensure the patient's lifetime daunorubicin equivalent does not exceed 460 mg/m^2 (see *Appendix G* for conversion table) or that their left ventricular ejection fraction (LVEF) does not drop below 45%. No intra-patient escalation of the alvocidib dose is permitted. *Consolidation - HiDAC* Consolidation therapy will consist of high-dose cytarabine (HiDAC) which may be initiated at the time of hematologic recovery or within 90 days of recovery. Consolidation therapy may start before complete hematologic recovery after discussion with the Medical Monitor. It is not anticipated that all patients will undergo Consolidation therapy. The HiDAC regimen will consist of 3 gm/m^2 IV every 12 hours on Days 1, 3, and 5 (a reduced dosage of 1.5 gm/m^2 is permitted for patients aged 60 to 65 years) (ie, a total of 6 doses over the 3-day regimen). The

	starting dose of HiDAC may be decreased pending discussion between the investigator and Medical Monitor. Patients who plan to receive chemotherapy-based consolidation should get a minimum of two cycles of Consolidation up to a maximum of four cycles. Patients proceeding to stem cell transplant for Consolidation should get one cycle of Consolidation, if possible, to ensure disease stability while transplant plans are being made.
Patient Population:	Patients enrolled in this study will be newly diagnosed with previously untreated AML excluding those patients with acute promyelocytic leukemia (APL-M3) or core-binding factor AML (CBF-AML) who meet all of the inclusion criteria and none of the exclusion criteria.
Inclusion Criteria:	To be eligible for participation in the study, patients must meet all of the following inclusion criteria: 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 with ≥20% bone marrow blasts based on histology or flow cytometry 3. Be newly diagnosed and previously untreated 4. Have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2 5. Have a serum creatinine level ≤1.8 mg/dL 6. Have an alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level ≤5 times upper limit of normal (ULN) 7. Have a total bilirubin level ≤2.0 mg/dL (unless secondary to Gilbert syndrome, hemolysis, or leukemia) 8. Have a left ventricular ejection fraction (LVEF) >45% by echocardiogram (ECHO) or multigated acquisition (MUGA) scan 9. 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 prior to study entry, for the duration of study participation, and for at least 6 months after the last dose of study drug (see *Section 4.5.3*). 10. Be able to comply with the requirements of the entire study. 11. 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 any previous treatment for AML 2. Diagnosed with APL-M3 or CBF-AML 3. Require concomitant chemotherapy, radiation therapy, or immunotherapy. Hydroxyurea is allowed up to the evening before starting (but not within 12 hours) of starting Induction therapy. 4. Received >200 mg/m^2 equivalents of daunorubicin (see *Appendix G* for conversion table)

	5. Have a peripheral blast count of >30,000/μL (may use hydroxyurea as in #3 above) 6. Have active central nervous system (CNS) leukemia 7. Have evidence of uncontrolled disseminated intravascular coagulation 8. Have an active, uncontrolled infection 9. Have other life-threatening illness 10. Have other active malignancies or diagnosed with other malignancies within the last 6 months, except nonmelanoma skin cancer or cervical intraepithelial neoplasia 11. Have mental deficits and/or psychiatric history that may compromise the ability to give written informed consent or to comply with the study protocol. 12. Are pregnant and/or nursing
Study Treatment:	**Study Drug Administration** *Induction – Alvocidib and 7+3* • *Days 1, 2, 3*: Alvocidib at a starting dose of 20 mg/m^2 as a 30-minute (±10 minutes) IV bolus followed by 30 mg/m^2 administered as a 4-hour (±15 minutes) IV infusion (this is the starting dose for Cohort 1; see table in this section for assigned alvocidib doses per treatment cohort) • *Day 4*: Drug-free day • *Days 5, 6, 7*: (7+3) Daunorubicin 60 mg/m^2 via IV bolus of up to 15 minutes' (±5 minutes) duration each day followed immediately by; • *Days 5, 6, 7, 8, 9, 10, 11*: Cytarabine 100 mg/m^2/day via continuous 24-hour IV infusion (±2 hours for infusion duration each day) *Reinduction – Alvocidib and 5+2* Patients **with** evidence of residual leukemia defined as >5% bone marrow blasts plus >10% cellularity, based on the Day 14 (±3 days) bone marrow may be eligible to start a second induction therapy with alvocidib plus 5+2 (Reinduction) at the discretion of the Investigator. • *Days 1, 2, 3:* Alvocidib at same dosage during Induction • *Day 4*: Drug-free day • *Days 5, 6:* (5+2) Daunorubicin 45 mg/m^2/day via IV bolus of up to 15 minutes' (±5 minutes) duration each day followed immediately by: • *Days 5, 6, 7, 8, 9:* Cytarabine 100 mg/m^2/day via continuous IV infusion (±2 hours for infusion duration each day) *Consolidation –HiDAC* Consolidation therapy will consist of high-dose cytarabine (HiDAC) which may be initiated at the time of hematologic recovery or within 90 days of recovery. Consolidation therapy may start before complete hematologic

recovery after discussion with the Medical Monitor. It is not anticipated that all patients will undergo Consolidation therapy.

- *Days 1, 3, 5:* HiDAC 3 gm/m^2 IV every 12 hours (1.5 gm/m^2 in patients aged 60 to 65 years) (ie, a total of 6 doses over the 3-day regimen); a lower starting dose may be permitted at discretion of Investigator and after discussion with the Medical Monitor.

Patients who plan to receive chemotherapy-based consolidation should get a minimum of two cycles of Consolidation up to a maximum of four cycles. Patients proceeding to stem cell transplant for Consolidation should get one cycle of Consolidation, if possible, to ensure disease stability while transplant plans are being made.

Dose Escalation of Alvocidib

Dose Levels[a]	Alvocidib Dosing		Number of Patients
	IV Bolus	Continuous IV × 4 hrs	
Cohort –1[b]	10 mg/m^2	15 mg/m^2	3-6
Cohort 1[c]	**20 mg/m^2**	**30 mg/m^2**	**3-6**
Cohort 2	30 mg/m^2	40 mg/m^2	3-6
Cohort 3	30 mg/m^2	50 mg/m^2	3-6
Cohort 4	30 mg/m^2	60 mg/m^2	3-6

a It is possible for additional and/or intermediate dose levels to be added during the cycle of the study.
b Dose level -1 represents treatment doses for patients requiring a dose reduction from the starting dose level. It will also serve as a lower dose level if ≥2 patients at the Starting Dose level experience a DLT.
c Starting Dose Level for first cohort of patients.

Once the MTD has been established, up to 20 additional patients will be enrolled at the MTD level for confirmation of safety as well as additional safety analysis and feasibility for determining MRD by dual methods (ie, MPFC and NGS) at a central laboratory.

Supportive Care

- Tumor Lysis Prevention and Treatment
  - 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 Induction (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.
- 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 Induction (optional in subsequent cycles) to be started at same time as initiation of IV hydration.
- Mandatory oral phosphate binder to be started at the same time as initiation of IV hydration during Induction (optional in subsequent cycles) unless contraindicated.

	• Evaluation of laboratory indicators of tumor lysis syndrome (TLS) during Induction: 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 o Monitor tumor lysis labs at the start of treatment and throughout the treatment cycle according to the schedule outlined in *Section 4.4.1.1.* [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.] o Monitor fibrinogen levels at baseline and then as clinically indicated o Patients who are determined to be at intermediate- or high-risk for TLS should be considered for rasburicase prophylaxis according to institutional standards • Infection Prevention o 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 at the start of chemotherapy. Alternative prophylactic antibiotic and antiviral therapy is left to the discretion of the treating physician and institutional standards. o Antifungal prophylaxis to be administered according to each institution's standard of care o Routine growth factor support is not allowed ≤Day 35 unless otherwise discussed with the Medical Monitor. Growth factor support can be given at the discretion of the Investigator >Day 35 of Induction/Reinduction therapy in the presence of life threatening infection with ongoing neutropenia. Growth factor support during Consolidation therapy may be administered according to each institution's standard of care. 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.
Safety Evaluations:	Through escalation of the dosage of alvocidib, the MTD and any DLTs will be defined. Safety and tolerability will also 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 [38] 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 observed values and changes from baseline values.
Efficacy Evaluations:	Objective response to treatment will be determined using the remission definitions detailed in the 2017 European LeukemiaNet Recommendations in AML (see *Appendix E*) at hematologic recovery (ie, ANC >1000/μL and platelet count >100,000/μL) or Day 50 (±3 days), counted from Day 1 of Induction therapy, whichever occurs first. If a patient receives a second induction cycle (or 'Reinduction') of alvocidib and 5+2, then the patient will undergo response assessment at the time of hematologic recovery (ie, ANC >1000/μL and platelet count >100,000/μL) or Day 60 (±3 days), counted from Day 1 of Induction therapy, whichever occurs first. • CR_{MRD-} = CR without MRD • CR = Complete Remission (including patients with morphologic CR who are MRD+ and cytogenetic and/or molecular marker positive) • CR_i = CR with incomplete hematologic recovery • MLFS = Morphologic leukemia-free state • PR = Partial Remission • SD = Stable Disease • PD = Progressive Disease • Overall Complete Remission Rate (patients with a best objective response of CR_{MRD}, CR, CR_i or MLFS) • Overall Remission Rate (patients with a best objective response of CR_{MRD}, CR, CR_i, MLFS or PR) • Overall Survival • Relapse-free Survival • Duration of Remission Additional bone marrow assessments may be obtained per Investigator discretion.
Pharmaco-dynamic Evaluations:	Any evidence of MRD will be determined using standardized techniques (ie, MPFC and NGS) and potential biomarkers including, but not limited to, MCL-1 dependency will also be assessed using bone marrow aspirates and peripheral blood samples.
Study Duration:	The study is expected to take 12-18 months to enroll up to 40 patients.

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.

1.1.1 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 NOXA priming can predict the clinical activity of alvocidib in AML patient samples and suggests an important role for MCL-1 activity in predicting alvocidib activity. MS1 and TMS1 are alternatives to NOXA that also measure MCL-1 dependency in addition to other potential molecular (genetic) targets.

1.1.2 Newly Diagnosed and Previously Untreated AML

Genetic abnormalities are the prevailing prognostic factors in determining response to treatment [10]. A number of studies have investigated the relative contribution of genetic and clinical variables to prediction of event-free survival (EFS) and overall survival (OS). Results from conventional cytogenetics and from certain mutational screenings are now routinely employed in clinical practice following recommendations by the European LeukemiaNet group in 2017.

The presence of specific genomic aberrations accounts for about two thirds of variability in predicting disease response in patients with AML; the remaining third is a combination of demographic, clinical, and treatment variables [10]. Advances have been made in incorporating these key data into models to aid physicians in predicting whether a given set of covariates conveys a longer remission or life expectancy compared to another patient with a different set of covariates. However, the accuracy of these models in correctly pinpointing the likelihood of response hovers around 75%. Along with the need to identify additional pretreatment prognostic factors that can address the remaining quarter of patients in whom response is likely, there is hope that evaluation of post-treatment events such as the presence of minimal residual disease (MRD) will further boost the percentage of likely responders.

There are different methods one can use to assess the presence of MRD, with each methodology varying in the proportion of patients to whom it can be applied and in its sensitivity to detect MRD. By integrating the evaluation of baseline factors together with the assessment of MRD, risk assessment should improve to better direct post-remission therapy.

1.2 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 has investigated the preclinical combination of alvocidib with agents used in the 7+3 regimen. In AML cell lines, single-agent IC_{50} values of alvocidib, cytarabine, and daunorubicin ranged from 2.2 nM to as high as 567 nM in viability assays. Apoptosis assays (Caspase-Glo) revealed modest induction with single agent cytarabine, and good induction with single agent daunorubicin or alvocidib. In combination therapy, however, very strong synergy (ie, a more than two-fold enhanced induction of apoptosis) has been observed (or

achieved) in some treatment groups. Alvocidib treatment reduced the expression of MCL-1 protein and mRNA in a time- and concentration-dependent fashion in AML cells. This has also been observed in the 7+3 treatment context. In an MV4-11 xenograft model, single-agent treatment with daunorubicin or cytarabine resulted in 21.1% and 48.5% tumor growth inhibition (%TGI), respectively; whereas treatment with 1.25 mg/kg of alvocidib yielded 60.0% TGI. The combination of all three agents—alvocidib, cytarabine and daunorubicin—resulted in even greater tumor regression with a TGI of 116.2%.

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 satisfactory survival rates for the majority of AML patients.

2. DRUG INFORMATION – ALVOCIDIB

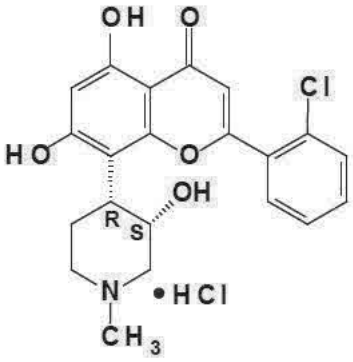
A comprehensive review of Alvocidib is contained in the Investigator's Brochure provided by the Sponsor. This document should be reviewed prior to initiating the study.

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).

2.4 Mechanism of Action

Alvocidib is a potent cyclin-dependent kinase (CDK) inhibitor with selectivity for CDKs 9, 1, 2, 4 and 7 [11, 12, 13]. The greatest inhibition (K_i of 3 nM) was observed with CDK 9 [14]. 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 [15, 16, 17]. 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 [18, 19]. 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 [20].

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 [21].

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 [22].

2.5 Preclinical Studies

2.5.1 In Vitro/In Vivo Studies

Alvocidib (2 to 430 nM) demonstrated cyclin-dependent kinase (CDK) selective inhibition when tested on a panel of recombinant kinases [11, 12, 13]. In particular, CDK 9 is involved in the regulation of transcription by phosphorylating ribonucleic acid polymerase II (RNA pol II) and is inhibited to the greatest extent (3 nM) [14]. Consistent with the CDK 9 inhibition, alvocidib (50 to 200 nM) significantly inhibited the uptake (up to 80%) of [3H]-uridine incorporation into total ribonucleic acid (RNA) compatible with inhibition of RNA transcription as a primary mechanism of action [23].

Alvocidib inhibited the proliferation of cell lines from a large panel of histologically distinct hematological and solid tumors at submicromolar concentrations ranging from 7 nM (PC3 human prostate) to 182 nM (K562 human chronic myelogenous leukemia). Alvocidib also induced dose dependent apoptosis in B-cell chronic lymphocytic leukemia (B-CLL) cells at drug concentrations ranging from 10 to 100 nM [24, 25]. The induction of apoptosis correlated best with depletion of the antiapoptotic proteins myeloid cell leukemia-1 (MCL-1) and X-linked inhibitor of apoptosis (XIAP) [15, 17].

Chronic lymphocytic leukemia cells are nonproliferating and dependent on the continuous expression of antiapoptotic proteins. As a result, reduction of antiapoptotic proteins by alvocidib may in part account for the drug-induced apoptotic response.

Significant antitumor activity was observed in 6 human leukemia xenograft models in mice (EOL-1 and ML-2 acute myeloid leukemia [AML], Ramos nonHodgkin's lymphoma, SUDHL-4 follicular lymphoma, HL-60 promyelocytic leukemia, and L363 multiple myeloma). In the SUDHL-4 and HL-60 studies, optimal activity was observed with daily × 5 bolus intravenous (IV) or intraperitoneal administration of 7.5 mg/kg alvocidib that gave peak plasma levels of 7 µM, followed by a progressive decline to approximately 100 nM in 8 hours [26]. In contrast, continuous infusion of alvocidib for 3 days demonstrated only modest activity. This infusion resulted in plasma levels of approximately 420 nM; these levels exceeded the in vitro 50% inhibitory concentration (IC_{50}), 20 to 200 nM, for most cell lines. This observation is consistent with the finding that alvocidib binds strongly to plasma proteins. These data also indicate that protein binding can be overcome with higher doses of alvocidib that achieve micromolar concentrations for short duration.

Alvocidib has enhanced antitumor activity when combined with standard of care agents in nonclinical models of AML. Due to its MCL-1-targeting activity, cells treated with alvocidib are in a primed state to undergo apoptosis which can be further exploited when followed by an agent known to induce apoptosis. Indeed, nonclinical data in AML strongly suggest that cytarabine and mitoxantrone are highly synergistic when preceded by alvocidib treatment. These data support the clinical regimen combining alvocidib, cytarabine, and mitoxantrone (ACM) [27].

Another study was designed to model the '7+3' clinical regimen in an animal model to determine if alvocidib could enhance the efficacy of cytarabine and daunorubicin in the MV-4-11 model for AML. Mice were given two 'cycles' of alvocidib, cytarabine, and daunorubicin (ACD), in which they were dosed for two days with alvocidib, one day with cytarabine and daunorubicin, followed by two additional days with cytarabine alone. Following these two treatment cycles, tumor volumes and body weights were observed until volumes neared 1500 mm^3. The ACD regimen modeled in this xenograft study demonstrated superior efficacy when compared to either cytarabine, daunorubicin, or the combination of the two drugs. The full drug combination (ACD) was tolerated well at the lower alvocidib dose level (1.25 mg/kg) and tolerated moderately at the higher dose level (2.5 mg/kg) with minor body weight loss observed. In the ACD regimen, alvocidib significantly enhanced tumor growth inhibition [28].

Alvocidib demonstrated only minor activity when evaluated in vivo in various solid tumor models.

2.5.2 Safety Pharmacology

[redacted]

2.5.3 Nonclinical Absorption, Distribution, Metabolism and Excretion

[redacted]

2.5.4 Animal Toxicology

[redacted]

[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 hematologic malignancies as well as in solid tumors. 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) [29]. 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 (historically known as 'FLAM', but referred to as 'ACM' in this study). 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 [30]	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 [31]	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 [32]	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 [33]	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 [34]	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 [35] ***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 [36]	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 [37]	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 Phase 2 Studies in Patients with Newly Diagnosed and Previously Untreated AML

Multiple Phase 2 studies have been conducted using ACM (FLAM) in patients with newly diagnosed nonfavorable (ie, high)-risk AML. In most studies, nonfavorable-/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).

Key study parameters are provided in *Table 2* followed by summaries of safety for each study.

Table 2: Alvocidib/FLAM in Newly Diagnosed Poor-risk AML

Indication (Study) [Ref]	Phase	Number of Patients Total/Evaluable	Alvocidib Administration (other agents)	Number of CRs
Newly diagnosed poor risk acute myelogenous leukemia (NCI-7845, J0669) [33] Completed	2	60/57	Alvocidib: 50 mg/m^2/d by 1hr IV on Days 1-3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 by IV over 60-120 min on Day 9	30 CR
Newly diagnosed poor risk acute myelogenous leukemia (NCI-8237J0856) [36] Completed	2	39/39 39/39	Arm A: Alvocidib 50 mg/m^2/d by 1hr IV on Days 1-3 Ara-C: 2 gm/m^2/72h by CIV on Days 6-8; Mitoxantrone: 40 mg/m^2 by IV over 60-120 min on Day 9 Arm B: Alvocidib 30 mg/m^2 by 30-min infusion followed by 40 mg/m^2 by 4h infusion on Days 1-3; Ara-C and mitoxantrone as in Arm A	24 CR 29 CR
Newly diagnosed poor risk acute myelogenous leukemia (NCI-8972, J1101) [37] Completed	2	109/109 56/56	Randomized study of: Arm A: Alvocidib 50 mg/m^2/d by 1hr IV on Days 1-3; Ara-C: 2 gm/m^2/72h by CIV on Days 6-8 Mitoxantrone: 40 mg/m^2 by IV over 60-120 min on Day 9 Arm B: 7+3 Ara-c: 100 mg/m^2/day by IV infusion on Days 1-7 Daunorubicin 90 mg/m^2/d by IV over 30-60 minutes on Days 1-3	76 CR 26 CR

2.6.2.1 Summary of Safety in NCI-7845 (J-0669) FLAM in Newly-diagnosed Poor-risk AML

All-grade Tumor Lysis Syndrome was observed in 19/45 (42%), with one patient expiring due to disseminated intravascular coagulation (DIC) and multiorgan failure. Treatment-related mortality (TRM) at 30 days was observed in 2 patients (4%), with one patient each with TLS/DIC/multiorgan failure and fungal infection. TRM at 60 days was observed in 2 additional patients (4%), both died from sepsis. Adverse events Grade ≥3 included: 11% GI mucositis, 11% reversible SV arrhythmia with sepsis, 7% sepsis, 4% oral mucositis, and 4% diarrhea.

2.6.2.2 Summary of Safety in NCI-8237 (J-0856) FLAM in Newly-diagnosed Poor-risk AML

The rate of all-grade Tumor Lysis Syndrome was equivalent on both arms (9%), as well as death from any cause at 60 days (8%, with 3 patients on each arm). Adverse events Grade ≥3 included: 50% febrile neutropenia, 35% infections, 14% nausea, 6% cardiac-related events, and 6% oral/GI mucositis.

The time from initiation of therapy to hematologic recovery was similar to previous FLAM studies, with median time to ANC over 0.5×10^9/L being 33 days (range 22-71 days) and platelets over 50×10^9/L being 30 days (range 21-80 days) for both arms.

2.6.2.3 Summary of Safety in NCI-8972 (J-1101) FLAM in Newly-diagnosed Poor-risk AML

In this comparison of FLAM vs. 7+3, adverse events were similar in both arms. Comparative adverse events Grade ≥3 included (FLAM, 7+3): febrile neutropenia (48%, 45%), infection (35%, 38%), hepatic dysfunction (21%, 23%), and GI events (11%, 9%).

Based on previous observations of Tumor Lysis Syndrome in prior AML studies of FLAM, preventive measures were included in this study including the use of allopurinol, phosphate binder, hydration, and rasburicase was allowed per institutional policy for hyperuricemia. The rates of TLS were similar between FLAM and 7+3 (FLAM: 8% versus 7+3: 7%). Treatment-related mortality was also similar between the arms at 30 days: FLAM: 5% versus 7+3: 2%, or at 60 days FLAM: 10% versus 7+3: 4%. The majority of early deaths (8/11 patients) on FLAM 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 the development of alvocidib in combination with cytarabine and daunorubicin regimen (7+3) in patients with newly diagnosed and previously untreated AML. As previously noted, these patients have a poor prognosis and new therapeutic options are needed. The combination of alvocidib plus cytarabine and daunorubicin is being explored as one of the regimens that can be considered for these patients.

NOXA binds and deactivates MCL-1. Early studies have suggested that NOXA priming 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 1 study, in part, to explore the ability of MCL-1 activity to predict which patients will respond favorably to alvocidib treatment when administered along with cytarabine and daunorubicin. Any effects of alvocidib on MRD status will also be evaluated.

While both the bolus as well as hybrid dosing regimens of alvocidib 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 [36].

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) [34]. 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.

2.8 Summary of Risk and Benefits

There have been no clinical studies conducted using alvocidib in combination with cytarabine and daunorubicin; however, the safety profile for alvocidib in combination with cytarabine and mitoxantrone has been well-described in eight clinical studies in patients with AML (see *Table 1*) and appears to be acceptable in patients with nonfavorable disease characteristics. 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 alvocidib. Treatment-related mortality (TRM) for patients treated with alvocidib/FLAM is approximately 8-10%, with a range up to 28% in Study 6 in 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

Primary Objective:

- To determine the safety and tolerability including the maximum tolerated dose (MTD) and dose-limiting toxicities (DLTs) of alvocidib when administered over a range of doses on Days 1-3 followed by cytarabine/daunorubicin (7+3) on Days 5-11 in adults with newly diagnosed and previously untreated AML

Secondary Objectives:

- To observe patients for any evidence of antileukemic activity of alvocidib plus 7+3 using the 2017 ELN response criteria
- To establish the Recommended Phase 2 Dose (RP2D) for future studies with alvocidib in combination with 7+3

Exploratory Objective***s***:

- To assess levels of minimal residual disease (MRD) using standardized techniques (ie, multiparametric flow cytometry [MPFC] and next generation sequencing [NGS]) and evaluate other potential biomarkers including, but not limited to, MCL-1 dependency.

4. INVESTIGATIONAL PLAN

4.1 Overall Study Design

This is an open-label, multicenter, dose-escalation, safety and biomarker prediction study of alvocidib followed by 7+3 therapy in adults with newly diagnosed and previously untreated AML.

Induction – Alvocidib and 7+3

The starting dose of alvocidib will be 20 mg/m^2 as a 30-minute intravenous (IV) bolus followed by 30 mg/m^2 over 4 hours as an IV infusion administered daily on Days 1-3 of Induction. Patients will have a one day drug holiday (Day 4) before initiation of the 7+3 regimen. Beginning on Day 5, cytarabine will be administered as a 100 mg/m^2/day continuous IV infusion for seven consecutive days (Days 5-11) plus daunorubicin administered at a dosage of 60 mg/m^2 via IV bolus of up to 15 minutes' duration on Days 5-7.

Escalation of the alvocidib dose will follow a standard 3+3 design with sequential cohorts of three patients treated with incrementally higher doses of alvocidib until a DLT is observed and the MTD is established. Three patients within a dose cohort may be enrolled without a waiting period between patients. Escalation to the next higher dose level will not occur until all patients within the current dose cohort have undergone full safety assessments (including count recovery or disease assessment).

If 1 of 3 patients in a cohort experiences a DLT, up to 3 additional patients will be treated at that dose level. If all patients in the expanded cohort have undergone full safety assessments (including count recovery or disease assessment) with no additional DLTs observed, the alvocidib dose will be escalated in a new cohort of 3 patients. If 2 or more of 3-6 patients at a given dose level experience a DLT during the first cycle, then the MTD will have been exceeded and up to a total of 6 patients will be treated at the previous lower dose level. If 0 or 1 of 6 patients experiences a DLT at this previous lower dose level, this dose will be declared the MTD.

The MTD is defined as the dose at which ≤1 of 6 patients experience a DLT during Cycle 1 with the next higher dose having at least 2 of 3-6 patients experiencing a DLT during Cycle 1.

The period of DLT determination for alvocidib and 7+3 ends at the start of consolidation therapy. However, any AEs that occur during Reinduction and meet DLT criteria will be taken into consideration when determining whether to escalate the dose of alvocidib and when defining the MTD.

Once the MTD has been established, up to 20 additional patients will be enrolled at the MTD level for confirmation of safety as well as additional safety analysis and feasibility for determining MRD by dual methods (ie, MPFC and NGS) at a central laboratory.

Disease Assessment

On Day 14 (±3 days), unless medically contraindicated, peripheral blood and bone marrow samples will be collected for disease assessment including MRD.

Patients with **no** evidence of residual leukemia (≤5% bone marrow blasts plus ≤10% cellularity) will be monitored weekly and will undergo response assessment at the time of hematologic recovery (ie, absolute neutrophil count (ANC) >1000/µL and platelet count >100,000/µL) or Day 50 (±3 days), counted from Day 1 of Induction therapy, whichever occurs first. At this time, the patient may continue to Consolidation therapy with HiDAC.

Reinduction – Alvocidib and 5+2

Patients **with** evidence of residual leukemia (>5% bone marrow blasts plus >10% cellularity) may be eligible for a second induction cycle (or 'Reinduction') with alvocidib and 5+2 at the discretion of the Investigator. The dose of alvocidib will remain the same, but the 7+3 regimen will be changed to 5+2: cytarabine, 100 mg/m^2/day, as a continuous IV infusion on Days 5-9 plus daunorubicin administered at a dosage of 45 mg/m^2 via IV bolus of up to 15 minutes' duration on Days 5 and 6.

Should a patient undergo Reinduction therapy, they will be monitored weekly and will undergo response assessment at the time of hematologic recovery (ie, ANC >1000/µL and platelet count >100,000/µL) or Day 60 (±3 days), counted from Day 1 of Induction therapy, whichever occurs first. Upon documented hematologic recovery, the patient may continue to Consolidation therapy with HiDAC. Additional bone marrow assessments may be obtained per investigator discretion.

Investigators are permitted to administer intrathecal (IT) chemotherapy per institutional protocols after Induction (preferably during marrow aplasia), but before starting Consolidation therapy. Additional doses of IT chemotherapy may be administered after discussion with the Medical Monitor.

During both Induction and Reinduction, if necessary, care must be taken to ensure the patient's lifetime daunorubicin equivalent does not exceed 460 mg/m^2 (see *Appendix G* for conversion table) or that their left ventricular ejection fraction (LVEF) does not drop below 45%.

No intra-patient escalation of the alvocidib dose is permitted.

Consolidation – HiDAC

Consolidation therapy with HiDAC may be initiated at the time of hematologic recovery or within 90 days of recovery. Consolidation therapy may start before complete hematologic recovery after discussion with the Medical Monitor. It is not anticipated that all patients will undergo Consolidation therapy.

The HiDAC regimen will consist of 3 gm/m^2 IV every 12 hours on Days 1, 3, and 5 (a reduced dosage of 1.5 gm/m^2 is permitted for patients aged 60 to 65 years) (ie, a total of 6 doses over the 3-day regimen). The starting dose of HiDAC may be decreased pending discussion between the investigator and Medical Monitor.

Patients who plan to receive chemotherapy-based consolidation should get a minimum of two cycles of Consolidation up to a maximum of four cycles. Patients proceeding to stem cell transplant for Consolidation should get one cycle of Consolidation, if possible, to ensure disease stability while transplant plans are being made.

4.2 Patient Population

Patients enrolled in this study must be newly diagnosed with previously untreated AML excluding those patients with acute promyelocytic leukemia (APL-M3) or core-binding factor AML (CBF-AML).

4.2.1 Number of Patients

A sufficient number of patients will be screened in order to obtain up to 40 eligible and evaluable patients for dosing. Any patient who withdraws from the study before completing Induction/Reinduction therapy will be replaced unless that patient is withdrawn due to toxicity or has experienced a DLT.

4.2.2 Inclusion Criteria

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

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 with ≥20% bone marrow blasts based on histology or flow cytometry
3. Be newly diagnosed and previously untreated
4. Have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) ≤2
5. Have a serum creatinine level ≤1.8 mg/dL
6. Have an alanine aminotransferase (ALT) and aspartate aminotransferase (AST) level ≤5 times upper limit of normal (ULN)

7. Have a total bilirubin level ≤2.0 mg/dL (unless secondary to Gilbert syndrome, hemolysis, or leukemia)
8. Have a left ventricular ejection fraction (LVEF) >45% by echocardiogram (ECHO) or multigated acquisition (MUGA) scan
9. 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 prior to study entry, for the duration of study participation, and for at least 6 months after the last dose of study drug (see *Section 4.5.3*).
10. Be able to comply with the requirements of the entire study.
11. 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.)

4.2.3 Exclusion Criteria

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

1. Received any previous treatment for AML
2. Diagnosed with APL-M3 or CBF-AML
3. Require concomitant chemotherapy, radiation therapy, or immunotherapy. Hydroxyurea is allowed up to the evening before starting (but not within 12 hours) of starting Induction therapy.
4. Received >200 mg/m^2 equivalents of daunorubicin (see *Appendix G* for conversion table)
5. Have a peripheral blast count of >30,000/mm^3 (may use hydroxyurea as in #3 above)
6. Have active central nervous system (CNS) leukemia
7. Have evidence of uncontrolled disseminated intravascular coagulation
8. Have an active, uncontrolled infection
9. Have other life-threatening illness
10. Have other active malignancies or diagnosed with other malignancies within the last 6 months, except nonmelanoma skin cancer or cervical intraepithelial neoplasia
11. Have mental deficits and/or psychiatric history that may compromise the ability to give written informed consent or to comply with the study protocol.
12. Are pregnant and/or nursing

4.3 Study Treatments

4.3.1 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.

4.3.2 Study Drug Administration

Induction – Alvocidib and 7+3

- *Days 1, 2, 3*: Alvocidib at a starting dose of 20 mg/m^2 as a 30-minute (±10 minutes) IV bolus followed by 30 mg/m^2 administered as a 4-hour (±15 minutes) IV infusion (this is the starting dose for Cohort 1; see *Table 3* for assigned alvocidib doses per treatment cohort)
- *Day 4*: Drug-free day
- *Days 5, 6, 7*: (7+3) Daunorubicin 60 mg/m^2 via IV bolus of up to 15 minutes' (±5 minutes) duration each day followed immediately by;
- *Days 5, 6, 7, 8, 9, 10, 11*: Cytarabine 100 mg/m^2/day via continuous 24-hour IV infusion (±2 hours for infusion duration each day)

Reinduction – Alvocidib and 5+2

Patients **with** evidence of residual leukemia defined as >5% bone marrow blasts plus >10% cellularity, based on the Day 14 (±3 days) bone marrow will be eligible to start a second induction cycle (ie, Reinduction) with alvocidib plus 5+2. The second induction cycle with alvocidib plus 5+2 will be allowed per investigator discretion.

- *Days 1, 2, 3*: Alvocidib at same dosage as during Induction
- *Day 4*: Drug-free day
- *Days 5, 6:* (5+2) Daunorubicin 45 mg/m^2/day via IV bolus of up to 15 minutes' (±5 minutes) duration each day followed immediately by;
- *Days 5, 6, 7, 8, 9:* Cytarabine 100 mg/m^2/day via continuous IV infusion (±2 hours for infusion duration each day)

Consolidation – HiDAC

Consolidation therapy will consist of high-dose cytarabine (HiDAC) which may be initiated at the time of hematologic recovery or within 90 days of recovery. Consolidation therapy may start before complete hematologic recovery after discussion with the Medical Monitor. It is not anticipated that all patients will undergo Consolidation therapy.

- *Days 1, 3, 5:* HiDAC 3 gm/m^2 IV every 12 hours (1.5 gm/m^2 in patients aged 60 to 65 years) (ie, a total of 6 doses over the 3-day regimen); lower starting dose may be permitted at the discretion of the Investigator and after discussion with the Medical Monitor

Patients who plan to receive chemotherapy-based consolidation should get a minimum of two cycles of Consolidation up to a maximum of four cycles. Patients proceeding to stem cell transplant for Consolidation should get one cycle of Consolidation, if possible, to ensure disease stability while transplant plans are being made.

4.3.3 Dose Escalation of Alvocidib

Evaluation of the safety and efficacy of alvocidib and 7+3 in patients with newly diagnosed AML will occur in two steps:

- Step 1: Cohorts of 3-6 patients will receive escalating doses of alvocidib based on *Table 3* until a DLT is observed during Induction/Reinduction.

- Step 2: Confirmation of the MTD. Once the MTD has been established, up to 20 additional patients will be enrolled at the MTD level for confirmation of safety as well as additional safety analysis and feasibility for determining MRD by dual methods (ie, MPFC and NGS) at a central laboratory.

Table 3: Dose Escalation of Alvocidib

Dose Levels[a]	Alvocidib Dosing		Number of Patients
	IV Bolus	Continuous IV × 4 hrs	
Cohort –1[b]	10 mg/m^2	15 mg/m^2	3-6
Cohort 1[c]	**20 mg/m^2**	**30 mg/m^2**	**3-6**
Cohort 2	30 mg/m^2	40 mg/m^2	3-6
Cohort 3	30 mg/m^2	50 mg/m^2	3-6
Cohort 4	30 mg/m^2	60 mg/m^2	3-6

a It is possible for additional and/or intermediate dose levels to be added during the cycle of the study.
b Dose level -1 represents treatment doses for patients requiring a dose reduction from the starting dose level. It will also serve as a lower dose level if ≥2 patients at the starting dose level experience a DLT.
c Starting Dose Level for first cohort of patients.

4.3.3.1 Dose Escalation of Alvocidib – Step 1

Escalation of the alvocidib dose will follow a 3+3 design. Sequential cohorts of three patients will be treated with escalated doses until the MTD is established. In the absence of DLTs, the dose will be increased incrementally. Three patients within a dose cohort may be enrolled without a waiting period between patients. Escalation to the next higher dose level will not occur until all patients within the current dose cohort have undergone full safety assessments (including count recovery or disease assessment).

If 1 of 3 patients in a cohort experiences a DLT, up to 3 additional patients will be treated at that dose level. If all patients in the expanded cohort have undergone full safety assessments (including count recovery or disease assessment) with no additional DLTs observed, the alvocidib dose will be escalated in a new cohort of 3 patients. If 2 or more of 3-6 patients at a given dose level experience a DLT during the first cycle, then the MTD will have been exceeded and up to a total of 6 patients will be treated at the previous lower dose level. If 0 or 1 of 6 patients experiences a DLT at this previous lower dose level, this dose will be declared the MTD.

4.3.3.2 Identifying and Confirming the MTD – Step 2

The MTD is defined as the dose at which ≤1 of 6 patients experience a DLT during Cycle 1 with the next higher dose having at least 2 of 3-6 patients experiencing a DLT during Cycle 1.

Any AEs that occur during Reinduction and meet DLT criteria will be taken into consideration when determining whether to escalate the dose of alvocidib and when defining the MTD.

Once the MTD has been established, up to 20 additional patients will be enrolled at the MTD level for confirmation of safety as well as additional safety analysis and feasibility for determining MRD by dual methods (ie, MPFC and NGS) at a central laboratory.

4.4 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.

4.4.1 Management of Nonhematologic Toxicities

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

Adverse events that are moderate to severe in intensity (see *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.

4.4.1.1 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 Induction (optional for subsequent cycles including Reinduction). 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
- Mandatory oral phosphate binder to be started at the same time as initiation of IV hydration, unless contraindicated.
- Evaluation of laboratory indicators of tumor lysis syndrome (TLS) during Induction therapy. Evaluation of laboratory indicators of TLS may be adjusted for Reinduction and Consolidation therapy based on extent of tumor burden.
    - 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:
        - 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 daunorubicin and cytarabine (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

o For patients **NOT at high** risk for TLS:

- 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.
- If no evidence of TLS during first 24 hours, then monitor tumor lysis labs approximately every 6-8 hours for the remainder of alvocidib treatment and then every 12 hours after the completion of alvocidib and during cytarabine and daunorubicin.
- 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, investigators 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.

4.4.1.2 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. 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.

4.4.1.3 Nausea/Vomiting

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

4.4.1.4 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 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.

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

Routine growth factor support is not allowed ≤Day 35 unless otherwise discussed with the Medical Monitor. Growth factor support can be given at the discretion of the Investigator >Day 35 of Induction/Reinduction therapy in the presence of life-threatening infection with ongoing neutropenia. Growth factor support during Consolidation therapy may be administered according to each institution's standard of care.

4.4.2 Management of Hematologic Toxicities

Adverse events that are moderate to severe in intensity (see *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 Principal Investigator. All concomitant medications must be recorded on the appropriate CRF.

4.4.3 Dose-limiting Toxicities

Dose-limiting toxicities (DLTs) for alvocidib and 7+3 will be determined during **Induction and Reinduction** therapy, determined to be at least possibly related to study drug and defined as follows based on the NCI CTCAE version 4.03:

- Any Grade 4 nonhematologic toxicity considered at least possibly drug related
- Any Grade 3 nonhematologic toxicity considered at least possibly drug related and that does not resolve to ≤Grade 2 within 48 hours, with the following exceptions:
    - Grade 3 diarrhea, mucositis, nausea or vomiting will be considered dose-limiting only if resolution to <Grade 2 (including use of supportive care) requires more than 7 days
- Grade 4 cytopenias lasting ≥50 days from the start of the cycle in the absence of residual leukemia if subject received one cycle of induction

- Grade 4 cytopenias lasting ≥60 days in the absence of residual leukemia if subject received 2 cycles of induction
- Grade 3 or 4 tumor lysis syndrome or related electrolyte disturbances (eg, hyperkalemia, hypophosphatemia, hyperuricemia) that do not resolve to ≤Grade 2 within 14 days
- ≥Grade 3 elevations in creatinine that do not resolve to ≤Grade 2 within 7 days
- Any AST and ALT elevation ≥5 ×ULN accompanied by serum bilirubin levels >2 ×ULN, regardless of duration
- Any Grade 5 toxicity that is not clearly and incontrovertibly related to the underlying disease or extraneous causes
- Anorexia, alopecia, fever, neutropenic fever, and infections of any grade should not be designated as DLTs since they are known and expected toxicities associated with the study drugs.

4.4.4 Dose Modifications

During Induction/Reinduction, there will be no dose reductions for alvocidib or cytarabine. Unless an elevation in total bilirubin level is secondary to suspected Gilbert syndrome, hemolysis (as reflected by an increase in indirect bilirubin level), or hepatic leukemic infiltration, the dose of daunorubicin must be reduced by 25% per day per dose if the patient's total direct bilirubin value is ≥1.2 mg/dL and ≤3 mg/dL, and must be reduced 50% per day per dose if the patient's total direct bilirubin value is >3 mg/dL on the day of planned daunorubicin administration. Bilirubin adjustment should be based on direct measurement. Patients with serum creatinine concentrations >3 mg/dL should receive 50% of the planned dose. Daunorubicin 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 their left ventricular ejection fraction (LVEF) drops below 45%.

The HiDAC dose may be modified during Consolidation at the discretion Investigator.

4.5 Concomitant Medications and Therapies

4.5.1 Previous Therapies

During Screening, patients will be asked about 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.

Patients will not be enrolled into the study if they have received >100 mg/m^2 equivalents of daunorubicin (see *Appendix G* for conversion table).

4.5.2 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.

4.5.2.1 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.

Investigators are permitted to administer intrathecal (IT) chemotherapy per institutional protocols after Induction (preferably during marrow aplasia), but before starting Consolidation therapy. Additional doses of IT chemotherapy may be administered after discussion with the Medical Monitor.

Medications and procedures that are mandatory or permitted during the study are listed in *Section 4.4.1*.

4.5.2.2 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.

4.5.3 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 prior to study entry, for the duration of study participation, and for at least 6 months after the last dose of study drug. The following are considered effective contraceptives: (1) oral contraceptive pill; (2) condom plus spermicide; (3) diaphragm plus spermicide; (4) abstinence; (5) patient or partner surgically sterile; (6) patient or partner more than 2 years post-menopausal; or (7) injectable or implantable agent/device.

4.6 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 Principal Investigator). In the event of a significant deviation from the protocol due to an emergency, accident or error, the Principal Investigator 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 Principal Investigator and the Sponsor.

4.7 Other Precautions

Dose adjustments for nonhematologic and hematologic toxicities and laboratory abnormalities will be made according to *Section 4.4.1* and *Section 4.4.2*, respectively.

5. ON-STUDY CLINICAL AND LABORATORY EVALUATIONS

See *Appendix A* - Schedule of Events

5.1 Screening (Within 2 Weeks Prior to First Dose of Alvocidib)

5.1.1 Within 2 Weeks Prior to First Dose

- Obtain written informed consent
- Perform bone marrow biopsy and/or aspiration and collect peripheral blood for diagnosis, cytogenetic profiling, MPFC, molecular testing, and assessment of other potential biomarkers
- Record results of molecular testing from bone marrow or peripheral blood that was collected as standard of care
- Collect and document a complete medical history including pathological confirmed diagnosis of AML by WHO criteria, confirmation of newly diagnosed and previously untreated disease, 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 12-lead electrocardiogram (ECG)
- Perform echocardiogram (ECHO) or multigated acquisition (MUGA) scan
- Perform chest radiograph (may omit, if performed/obtained within 30 days prior to anticipated first dose)
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements taken within the past 2 weeks

5.1.2 Within 72 Hours Prior to First Dose of Alvocidib

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

- 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
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements
- Lumbar puncture for those patients with clinical symptoms suspicious of central nervous system involvement

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

5.2 Induction – Alvocidib and 7+3

5.2.1 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 completion of chemotherapy. Patients will be discharged after completion of chemotherapy once deemed clinically stable by the investigator.

5.2.1.1 Induction – At Least 10 Hours Prior to First Dose

Perform the following procedures at least 10 hours prior to starting Induction therapy on Day 1 (unless otherwise stated):

- Abbreviated physical examination including weight (kg) for calculation of body surface area (BSA) (may be performed within 10 hours prior to first dose)
- Assess ECOG PS (*Appendix B*) (may be performed within 10 hours prior to first dose)
- 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 4.4.1*) (as clinically indicated during Reinduction)
    - 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. (To be performed if clinically indicated during Reinduction)
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements taken since screening (may be performed within 10 hours prior to first dose)

5.2.1.2 Induction, 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 prescription drugs, nonprescription drugs, and nutritional supplements

5.2.2 Induction, Day 1 – Dosing

- Monitor tumor lysis labs at the start of alvocidib and throughout the treatment cycle according to the schedule outlined in *Section 4.4.1.1*. [*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.
- Administer prophylactic antibiotics and antivirals, and antifungals according to *Section 4.4.1.4*
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.3 Induction – 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
  - Bilirubin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), alkaline phosphatase (ALP)
  - Tumor lysis labs as outlined in *Section 4.4.1.1* (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.
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.4 Induction – Weekly after Completion of Chemotherapy Regimen

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

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

5.2.5 Induction, Day 14 (±3 Days)

- Collect peripheral blood samples and perform a bone marrow biopsy on Day 14 (±3 days), unless medically contraindicated, for disease assessment including MRD and other potential biomarkers. Central laboratory assessment of MRD is to be done for disease assessment and treatment guidance.
    - Patients with **no** evidence of residual leukemia will be monitored weekly (as in *Section 5.2.4*) until hematologic recovery (go to *Section 5.2.6*)
    - Patients **with** evidence of residual leukemia (>5% bone marrow blasts plus >10% cellularity) may be eligible for a second Induction cycle (or 'Reinduction') with alvocidib and 5+2 at the discretion of the Investigator (go to *Section 5.3*).
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.6 Induction – At Hematologic Recovery or Day 50 (±3 days), Whichever Occurs First

- Patients will undergo response assessment including MRD via peripheral blood and bone marrow biopsy samples collected at the time of hematologic recovery (ie, absolute neutrophil count [ANC] >1000/μL and platelet count >100,000/μL) or Day 50 [±3 days], counted from Day 1 of Induction therapy, whichever occurs first. These same samples will also be used for assessment of other potential biomarkers. Central laboratory assessment of MRD is to be done for disease assessment and treatment guidance. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7-10 days.
- Additional bone marrow assessments may be obtained per Investigator discretion.
- Assess for AEs, including tumor lysis clinical symptoms
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3 Reinduction (if Day 14 [±3 Days] Positive Marrow)

Patients **with** evidence of residual leukemia (>5% bone marrow blasts plus >10% cellularity) may undergo Reinduction with alvocidib and 5+2 at the discretion of the Investigator. Care must be taken to ensure the patient's lifetime daunorubicin equivalent does not exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or that their LVEF does not drop below 45%.

Patients undergoing Reinduction will repeat the assessments in *Section 5.2.1* through *Section 5.2.5*.

5.3.1 Reinduction – At Hematologic Recovery or Day 60 (±3 days), Whichever Occurs First

- Following the second induction cycle (or 'Reinduction') of alvocidib and 5+2, patients will undergo response assessments including MRD via peripheral blood and bone marrow biopsy samples collected at the time of hematologic recovery (ie, ANC >1000/µL and platelet count >100,000/µL) or Day 60 (±3 days), counted from Day 1 of Induction therapy, whichever occurs first. These same samples will also be used for assessment of other potential biomarkers. Central laboratory assessment of MRD is to be done for disease assessment and treatment guidance. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7-10 days.
- Additional bone marrow assessments may be obtained per Investigator discretion.
- Assess for AEs, including tumor lysis clinical symptoms
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4 Consolidation – HiDAC

Consolidation therapy will consist of high-dose cytarabine (HiDAC) initiated at the time of hematologic recovery or within 90 days of recovery. Consolidation therapy may also be started before complete hematologic recovery after discussion with the Medical Monitor. It is not anticipated that all patients will undergo Consolidation therapy.

5.4.1 Consolidation, Day 1 – Just Prior to First Dose

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

- Abbreviated physical examination including weight (kg) for calculation of body surface area (BSA)
- Assess ECOG PS (*Appendix B*)

- Record vital signs (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*):
  - Hematology
  - Full serum chemistry panel
  - Fibrinogen level
- Collect urine or serum sample for β-hCG pregnancy test for females of child-bearing potential
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements administered in conjunction with an AE
- Assess for AEs

5.4.2 Consolidation, Day 1 – Dosing

- Administer recommended prophylactic antibiotics and antivirals, and antifungals according to each institution's standard of care
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.3 Consolidation – Daily during Hospitalization for HiDAC

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
  - Bilirubin, creatinine, ALT, AST, ALP
  - Tumor lysis labs (as clinically indicated)
  - Fibrinogen level (as clinically indicated)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.4 Consolidation – Weekly after Completion of HiDAC

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

- Collect blood for evaluation of laboratory parameters (*Appendix D*):
    - Hematology
    - Bilirubin, creatinine, ALT, AST, ALP
    - Tumor lysis labs (as clinically indicated)
    - Fibrinogen level (as clinically indicated)

5.4.5 Consolidation – Response Assessment

- After Consolidation cycles 2 and 4, patients will undergo response assessments including MRD via peripheral blood and bone marrow biopsy samples collected at the time of hematologic recovery (ie, ANC >1000/μL and platelet count >100,000/μL). These same samples will also be used for assessment of other potential biomarkers. Central laboratory assessment of MRD is to be done for disease assessment and treatment guidance. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7-10 days.
- Additional bone marrow samples may be requested at the discretion of the Investigator.
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.4.6 Consolidation – Cycles 2 through 4

Patients who plan to receive chemotherapy-based consolidation should get a minimum of two cycles of Consolidation up to a maximum of four cycles. Patients proceeding to stem cell transplant for Consolidation should get one cycle of Consolidation, if possible, to ensure disease stability while transplant plans are being made.

Each cycle will include the assessments listed in *Section 5.4.1* through *Section 5.4.5*.

5.5 End of Study Assessments

If, at any time, a patient discontinues study treatment, a visit should be scheduled as soon as possible and within 14 days of the last dose of study drug or within 14 days of the decision to discontinue study treatment. If the decision to withdraw the patient occurs at a regularly scheduled visit, that visit may become the End of Study visit rather than having the patient return for an additional visit.

- Complete 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 (as clinically indicated)
  - Fibrinogen level (only required if clinically indicated)
- Urinalysis
- Perform bone marrow biopsy and/or aspiration (if not done in the preceding 30 days) for determination of disease status including MRD and assessment of other potential biomarkers. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7-10 days.
- 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

5.6.1 *Safety Follow-up (30 Days Post Last Dose)*

Patients must have a safety evaluation 30 days after the last dose of study drug. The following assessments will be performed:

- Assess for AEs
  - Ongoing AEs must be followed clinically until the event is resolved, deemed stable, or the patient starts another treatment for their disease
- Record only those medications (prescription, nonprescription, and nutritional supplements) administered in conjunction with an AE, as well

as any antineoplastic therapies initiated since discontinuation of study drug.

5.6.2 Long-term Follow-up (Starting 30 Days Post End of Study out to 2 Years)

Additionally, all study patients will be attempted to be contacted by phone, if possible, every 6 months to assess for date of death, date of stem cell transplant, date of relapse, or continued remission beginning the month after the patient completes the End of Study assessments to 24 months after Day of First Dose regardless of how many cycles a patient receives. When telephone contact is not possible, medical records will serve as the surrogate to assess for these outcomes.

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 Principal Investigator 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_{MRD-}, CR, CRi, MLFS, 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 ≥ Grade 3 toxicity considered at least possibly related to alvocidib.
- 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 Principal Investigator, 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, Principal Investigator, the Sponsor, or regulatory authority;
- Patient is lost to follow-up;
- Patient becomes pregnant while on study;
- Patient begins another treatment for their disease; or
- Patient death

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 starts another 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 *Section 8.4* through *Section 8.8* for reporting of adverse events. The pregnancy of any patient, or patient's partner, will be followed via monthly telephone calls until the birth of the child to term to record any birth defects/abnormalities.

7. CRITERIA FOR EVALUATION AND ENDPOINT DEFINITIONS

7.1 Safety Endpoints

Through escalation of the dosage of alvocidib, the MTD and any DLTs will be defined. Safety and tolerability will also 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 [38] will be used to assess the potential for development of TLS (see *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.

7.2 Efficacy Endpoints

Since the primary objective of this study is to determine safety and tolerability including of alvocidib when administered over a range of doses followed by cytarabine/daunorubicin (7+3) in adults with newly diagnosed and previously untreated AML, a formal efficacy analysis is not appropriate. However, any objective response to treatment will be determined using the remission definitions detailed in the 2017 European LeukemiaNet Recommendations in AML [10] after Induction (or Reinduction) (definitions provided in *Section 11.3.2*).

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 (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.

GRADE 1 – Mild:	Transient or mild discomfort; no limitation in activity; no medical intervention/therapy required.
GRADE 2 – Moderate:	Mild to moderate limitation in activity – some assistance may be needed; no or minimal medical intervention/therapy required.
GRADE 3 – Severe:	Marked limitation in activity, some assistance usually required; medical intervention/therapy required, hospitalizations possible.
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.
GRADE 5 – Fatal:	Results in death.

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*.

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, hospitalization or prolongation of hospitalization for 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 case report form 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 "Adverse Event" case report form 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. The Principal 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.

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 Principal Investigator's judgment using protocol-defined definitions. Necessary counter measures will also be reported on the appropriate case report form used to collect concomitant medications.

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

Any serious adverse event (SAE) or unexpected AE ≥NCI CTCAE Grade 3 that occurs during this study and up to 30 days after discontinuation of study drug must be reported to the Study Medical Monitor within 24 hours of the Principal 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 <u>any</u> AE leading to permanent discontinuation of study drug must also be reported to the Sponsor within 24 hours of the Principal Investigator's awareness of the event.

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

[redacted]

It is expected that the Principal Investigator will provide or arrange appropriate supportive care for the study patient. A patient experiencing a serious adverse event(s) should be followed clinically until the event is resolved, deemed stable or permanent, or the patient starts another treatment for their disease. All telephone and scanned/emailed reports must be followed with a written Serious Adverse Event (SAE) report form within 24 hours of the Principal 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 Principal 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 case report form.

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 Principal Investigator until the events resolve, are deemed stable or permanent, or the patient starts another treatment for their disease. Resolution means the patient has returned to his/her baseline state of health or the Principal Investigator does not expect any further improvement or worsening of the adverse event. The Principal 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 Principal Investigator which occur after the last scheduled visit, and are determined by the Principal 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***,*** or the patient starts 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 Principal 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 event leading up to the patient's death began within 30 days of the last administration of study drug.

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

8.8 Reporting Adverse Events to the Regulatory Authorities

The Sponsor will be responsible for reporting adverse events to the FDA as described in 21 CRF Section 312.32 (IND Safety Reports) and to other Regulatory Authorities according to local regulations.

In addition, the Principal Investigator is required by FDA regulations to notify the IRB promptly of all unexpected SAEs occurring at the investigator's study site. The Principal 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 Principal Investigator 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).

Cytarabine and daunorubicin, 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 Courante (CRO) 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, USP, or 5% Dextrose Injection, USP, 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 4.3.2*.

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 4.3.2*.

Daunorubicin Hydrochloride Injection, 5 mg/mL, is available as a deep red sterile liquid. The desired dose is withdrawn into a syringe containing 10 mL to 15 mL of 0.9% Sodium Chloride Injection, USP, and then injected into the tubing or side-arm in a rapidly flowing IV infusion of 5% Dextrose Injection, USP, or 0.9% Sodium Chloride Injection, USP. Daunorubicin hydrochloride should not be administered mixed with other drugs or heparin. Administer according to treatment schedule in *Section 4.3.2*.

Both cytarabine and daunorubicin 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 at USP controlled room temperature (ie, 20°C to 25°C [68°F to 77°F]) with permitted excursions between 2°C to 30°C (36°F to 86°F).
- Cytarabine (Ara-c) should be stored between 15° to 30°C (59° to 86°F).
- Daunorubicin (daunorubicin hydrochloride injection) should be stored at room temperature, 15° to 30°C (59° to 86°F) for up to 24 hours. Contains no preservative. Discard unused portion.

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 electronic Case Report Form (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 Case Report Forms) 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 Principal Investigator 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 Principal Investigator 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 SAS software version 9.4 or higher. In general, data summaries will be compiled by alvocidib dose level and overall and 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).

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

11.2 Sample Size

Given the design and primary objectives of this study, the sample size will be determined by the guidelines governing dose escalation/de-escalation and the identification of DLTs. As a result, the final sample size is not fixed and will not be determined by consideration of inferential analyses conducted via statistical hypothesis testing. It may be anticipated that the study sample size will be up to 40 patients. This is based on observing DLTs in 2 of 3 patients enrolled at the fourth dose level (Cohort 4) and declaring the third dose level as the MTD (3 patients each in the first and second dose levels, 6 patients in the third dose level during dose escalation, 3 patients at the fourth dose level, and an additional 20 patients at the third dose level for confirmation of safety as well as additional safety analysis and feasibility for determining MRD by dual methods [ie, MPFC and NGS] at a central laboratory). However, the actual number of patient enrolled may be slightly more or less depending on the actual pattern and frequency of DLTs.

11.3 Data Analyses

The MTD is defined as the largest dose for which fewer than 33% of patients experience a DLT. The MTD will be estimated by the highest alvocidib dose for which the empirical incidence of DLT during Cycle 1 was less than 33% in combination with 7+3.

Incidence rates of treatment-emergent adverse events 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) serious adverse events (SAEs).

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

The multivariate analysis and risk score prediction model by Montesinos and colleagues [38] will be used to assess the potential for development of TLS (see *Appendix F*).

11.3.1 Demographics and Baseline Characteristics

Demographics and baseline characteristics will be summarized by alvocidib dose level and overall using descriptive statistics and confidence intervals.

11.3.2 Efficacy Analyses

Objective response to treatment will be determined using the remission definitions detailed in the 2017 European LeukemiaNet Recommendations in AML [10] after Induction/Reinduction (for those patients not achieving a CR after Cycle 1) (see *Appendix E*).

- CR_{MRD-} = CR without MRD
- CR = Complete Remission (including patients with morphologic CR who are MRD+ and cytogenetic and/or molecular marker positive)
- CR_i = CR with incomplete hematologic recovery
- MLFS = Morphologic leukemia-free state
- PR = Partial Remission
- SD = Stable Disease
- PD = Progressive Disease

The numbers of overall complete remissions (patients with a best objective response of CR_{MRD}, CR, CR_i or MLFS), and overall remissions (patients with a best objective response of CR_{MRD}, CR, CR_i, MLFS or PR) will be summarized by observed rates and estimated 95% CIs. If there are sufficient numbers of patients achieving remissions for an analysis to be informative, logistic regression will be used to model the probability of achieving a remission as a function of the total alvocidib daily dose level (bolus dose amount + infusion dose amount) and biomarker prediction endpoints (eg, MRD).

Kaplan-Meier time-to-event analyses will be conducted on overall survival, relapse-free survival and duration of remission. Mortality (all causes) at 30 and 60 days will also be calculated. Cox proportional hazards models will be fit to these time-to-event endpoints with the total alvocidib daily dose level (bolus dose amount + infusion dose amount) and biomarker prediction endpoints (eg, MRD) serving as independent variables in the model.

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

11.3.3 Biomarker Prediction Endpoint Analyses

Summary of baseline biomarker values will include within– and between–treatment-arm descriptive statistics. If there are sufficient numbers of patients achieving remissions for an analysis to be informative, a logistic regression model will be fit to examine the relationship between baseline results and other potential biomarkers (including, but not limited to, MCL-1 dependency) and the independent binary variables complete remissions and overall remissions during the study. In addition, area under the curve (AUC) and 95% CI will be calculated (by alvocidib dose level) for the trapezoidal receiver operating characteristic curve. This value will quantify the ability of certain biomarkers (including, but not limited to, MCL-1 dependency) to predict complete/overall remissions.

11.3.4 Safety Analyses

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

- **Adverse Events** = Reported adverse event (AE) terms will be mapped to MedDRA preferred terminology. All reported events will appear in AE listings, however only treatment-emergent adverse events will be summarized. A treatment-emergent adverse event (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.

- **Clinical Laboratory Assessments** = Laboratory test results measured on a continuous scale and changes from baseline values will be summarized by visit and within alvocidib dose level using mean, standard deviation, median, minimum and maximum values. Categorical test results will be summarized within alvocidib dose level using shift tables. Additionally, for tests where NCI CTCAE, version 4.03, 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 alvocidib dose level 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 (SIV), 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 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 (COV) 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, 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 Principal Investigator 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 Good Clinical Practice (GCP). The Principal Investigator 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 Principal Investigator 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 case report forms.

15.2 Informed Consent

It is the ethical and legal responsibility of the Principal Investigator 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 Principal Investigator. The Principal Investigator 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 Principal Investigator (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 Principal Investigator. 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 Principal Investigator 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 Principal Investigator shall not participate in the decision, and, if an IRB/EC member, the written approval must indicate such non-participation. The Principal Investigator shall submit status reports to the IRB/IEC no less frequently than annually (when applicable).

The IRB/IEC must be notified by the Principal Investigator in writing of the interruption and/or completion of the study; the Principal Investigator 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 Principal Investigator will promptly report to the IRB/IEC all unanticipated problems involving risk to patients or others. The Principal Investigator 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.

19. REFERENCES

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APPENDIX A – SCHEDULE OF ACTIVITIES

	SCREENING		INDUCTION – ALVOCIDIB AND 7+3						
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 1-11)	Weekly - Post Treatment	Day 14 (±3)	At Hematologic Recovery or Day 50 (±3)
Bone marrow biopsy & peripheral blood sample	X[h]							X[r]	X[s]
Obtain study informed consent	X								
Record prior molecular testing results[v]	X								
Medical history[a]	X								
Physical examination+weight (kg)	X[u]		X[q]				X[q]		
Height (cm)	X								
ECOG performance status	X		X[k]				X		
Vital signs[b]	X			X[m]			X		
12-lead ECG	X								
ECHO or MUGA	X								
Chest radiograph[c]	X								
Concomitant medications	X[i]	X	X[j,k]	X	X[o]	X[o]	X[o]	X[o]	X[o]
Hematology[d]		X				X	X		
Full serum chemistry panel[d]		X							
Bilirubin, creatinine, ALT, AST, ALP[w]						X	X		
Urinalysis[d]		X							
Pregnancy test[e]		X		X[n]					
Lumbar puncture if suspicious of CNS involvement		X							
Review Inclusion/Exclusion criteria		X							
Determination of BSA			X[k]						
Pretreatment: IV hydration, allopurinol, oral phosphate binder			X[l]						
Monitor urine output frequently[f]			X		X	X			
Tumor lysis labs[d]				X	X	X	X		
Coagulation: fibrinogen				X	X[p]	X[p]	X[p]		
Prophylactic antibiotics and antivirals, antifungals					X				
Study treatment					X	X			
Assessment of AEs[g]					X	X	X	X	X[t]

Notes:

a Collect and document a complete medical history including pathological confirmed diagnosis of AML by WHO criteria, confirmation of newly diagnosed and previously untreated disease, and all other measures of disease and disease symptoms (eg, extramedullary disease)

b Vital signs to include: temperature, heart rate, systolic and diastolic blood pressures
c May omit if performed/obtained within 30 days prior to anticipated first dose
d See *Appendix D* – Laboratory Tests
e Collect urine or serum sample for β-hCG pregnancy test for females of child-bearing potential
f 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.
g Toxicities will be assessed according to the NCI CTCAE, version 4.03 (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.
h Bone marrow biopsy and/or aspiration and peripheral blood collected for diagnosis, cytogenetic profiling, MPFC, molecular testing, and assessment of other potential biomarkers.
i Including all prescription and nonprescription medications and nutritional supplements taken within the past 2 weeks
j Including all prescription and nonprescription medications and nutritional supplements taken since screening
k May be conducted within 10 hours prior to first dose
l 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) (*Section 4.4.1.1*). If, by Day 4, there is no evidence of tumor lysis syndrome, the hydration rate can be reduced to a maintenance level. Mandatory allopurinol orally each day of dosing for first cycle to be started at same time as initiation of IV hydration. Mandatory oral phosphate binder to be started at the same time as initiation of IV hydration, unless contraindicated.
m Measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest
n Required if screening pregnancy test was performed greater than 72 hrs prior to first dose
o To include only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE
p Only required if clinically indicated
q Abbreviated physical examination (AE- or symptom-directed exam)
r Bone marrow biopsy and peripheral blood sample collected, unless medically contraindicated, for disease assessment including MRD and assessment of other potential biomarkers. Central laboratory assessment of MRD is to be done for disease assessment and treatment guidance. Patients **with no** evidence of residual leukemia will be monitored weekly (as in *Section 5.2.4*) until hematologic recovery (*Section 5.2.6*). Patients **with** evidence of residual leukemia (>5% bone marrow blasts plus >10% cellularity) may be eligible for a second Induction cycle (or 'Reinduction') with alvocidib and 5+2 at the discretion of the Investigator (*Section 5.3*).
s Response assessment to include MRD via peripheral blood and bone marrow biopsy samples collected at the time of hematologic recovery (ie, absolute neutrophil count [ANC] >1000/μL and platelet count >100,000/μL) or Day 50 [±3 days], counted from Day 1 of Induction therapy, whichever occurs first. These same samples will also be used for assessment of other potential biomarkers. Central laboratory assessment of MRD is to be done for disease assessment and treatment guidance. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7-10 days. Additional bone marrow assessments may be obtained per Investigator discretion.
t Including tumor lysis clinical symptoms
u Complete physical examination including height (cm) and weight (kg)
v Record results of molecular testing from bone marrow or peripheral blood that was collected as standard of care
w Collect blood for evaluation of serum bilirubin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels

APPENDIX A – SCHEDULE OF ACTIVITIES

TESTS/PROCEDURES	REINDUCTION – ALVOCIDIB AND 5+2[a]					
	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 60 (±3)
Physical examination+weight (kg)	X[i,m]				X[m]	
ECOG performance status	X[i]				X	
Determination of BSA	X[i]					
Pretreatment: IV hydration, allopurinol, oral phosphate binder[b]	X					
Monitor urine output frequently[c]	X		X	X		
Concomitant medications[d]	X[i]	X	X	X	X	X
Vital signs[e]		X[j]			X	
Pregnancy test[f]		X				
Bilirubin, creatinine, ALT, AST, ALP[n]				X	X	
Tumor lysis labs[g]		X	X	X[l]	X	
Coagulation: fibrinogen		X	X[k]	X[k]	X[k]	
Study treatment			X	X		
Prophylactic antibiotics and antivirals, antifungals			X			
Bone marrow biopsy & peripheral blood sample						X[o]
Hematology[g]				X	X	
Assessment of AEs[h]			X	X	X	X[p]

Notes:

a On Day 14 (±3) of Induction therapy, patients **with** evidence of residual leukemia may undergo Reinduction with alvocidib and 5+2 at the discretion of the Investigator. Care must be taken to ensure the patient's lifetime daunorubicin equivalent does not exceeds 460 mg/m^2 (see *Appendix G* for conversion table) or that their LVEF does not drop below 45%.

b If clinically indicated during Reinduction, administer pretreatment IV hydration, oral allopurinol, and oral phosphate binder as during Induction.

c 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.

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

e Vital signs to include: temperature, heart rate, systolic and diastolic blood pressures

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

g See *Appendix D* – Laboratory Tests

h Toxicities will be assessed according to the NCI CTCAE, version 4.03 (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 May be conducted within 10 hours prior to first dose

j Measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest
k Only required if clinically indicated
l Or more frequently if clinically indicated
m Abbreviated physical examination (AE- or symptom-directed exam).
n Collect blood for evaluation of serum bilirubin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels
o Following Reinduction, response assessment to include MRD via peripheral blood and bone marrow biopsy samples collected at the time of hematologic recovery (ie, absolute neutrophil count [ANC] >1000/µL and platelet count >100,000/µL) or Day 60 [±3 days], counted from Day 1 of Induction therapy, whichever occurs first. These same samples will also be used for assessment of other potential biomarkers. Central laboratory assessment of MRD is to be done for disease assessment and treatment guidance. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7-10 days. Additional bone marrow assessments may be obtained per Investigator discretion.
p Including tumor lysis clinical symptoms.

APPENDIX A – SCHEDULE OF ACTIVITIES

	CONSOLIDATION – HiDAC[a]						
TESTS/PROCEDURES	Just Prior to 1st Dose	Dosing	Daily During HiDAC	Weekly - Post HiDAC	Response Assessment after Cycles 2 & 4	End of Study Assessments[l]	Follow Up[o, s]
Physical examination+weight (kg)	X[j]					X[m]	
Determination of BSA	X						
ECOG performance status	X					X	
Vital signs[b]	X[i]					X	
Pregnancy test[c]	X						
Hematology[d]	X		X	X		X	
Full serum chemistry panel[d]	X					X[g]	
Bilirubin, creatinine, ALT, AST, ALP [r]			X	X			
Concomitant medications[e]	X	X	X		X	X	X[p]
HiDAC		X	X				
Prophylactic antibiotics and antivirals, antifungals[f]		X					
Tumor lysis labs[d]			X[g]	X[g]			
Coagulation: fibrinogen	X		X[g]	X[g]		X[g]	
Bone marrow biopsy & peripheral blood sample					X[k]	X[n]	
12-lead ECG[g]						X	
ECHO or MUGA[g]						X	
Urinalysis[d]						X	
Assessment of AEs[h]	X	X	X		X	X	X[q]

Notes:

a HiDAC to be initiated at the time of hematologic recovery or within 90 days of recovery. Consolidation therapy may also be started before complete hematologic recovery after discussion with the Medical Monitor. Patients who plan to receive chemotherapy-based consolidation should get a minimum of two cycles of Consolidation up to a maximum of four cycles. Patients proceeding to stem cell transplant for Consolidation should get one cycle of Consolidation, if possible, to ensure disease stability while transplant plans are being made.

b Vital signs to include: temperature, heart rate, systolic and diastolic blood pressures

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

d See *Appendix D* – Laboratory Tests

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

f Recommended anti-infection prophylaxis to be administered according to each institution's standard of care.

g Only required if clinically indicated

h Toxicities will be assessed according to the NCI CTCAE, version 4.03 (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 Measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest

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

k After Cycle 2 and Cycle 4, assess response including MRD via peripheral blood and bone marrow biopsy samples collected at the time of hematologic recovery (ie, absolute neutrophil count [ANC] >1000/µL and platelet count >100,000/µL). These same samples will also be used for assessment of other potential biomarkers. Central laboratory assessment of MRD is to be done for disease assessment and treatment guidance. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7-10 days. Additional bone marrow assessments may be obtained per Investigator discretion.

l If, at any time, a patient discontinues study treatment, a visit should be scheduled as soon as possible and within 14 days of the last dose of study drug or within 14 days of the decision to discontinue study treatment. If the decision to withdraw the patient occurs at a regularly scheduled visit, that visit may become the End of Study visit rather than having the patient return for an additional visit.

m Complete physical examination including measures of disease and disease symptoms, eg, extramedullary disease.

n Perform bone marrow biopsy and/or aspiration (if not done in the preceding 30 days) for determination of disease status including MRD and assessment of other potential biomarkers. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7-10 days.

o Patients must have a safety evaluation 30 days after the last dose of study drug to assess any ongoing AEs and to record concomitant medications administered with an AE, as well as any antineoplastic therapies initiated since discontinuation of study drug.

p Record only those medications (prescription, nonprescription, and nutritional supplements) administered in conjunction with an AE, as well as any antineoplastic therapies initiated since discontinuation of study drug.

q Ongoing AEs must be followed clinically until the event is resolved, deemed stable, or the patient starts another treatment for their disease.

r Collect blood for evaluation of serum bilirubin, creatinine, alanine aminotransferase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP) levels

s ***All study patients will be attempted to be contacted by phone, if possible, every 6 months to assess for date of death, date of stem cell transplant, date of relapse, or continued remission beginning the month after the patient completes the End of Study assessments to 24 months after Day of First Dose regardless of how many cycles a patient receives. When telephone contact is not possible, medical records will serve as the surrogate to assess for these outcomes.***

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's Common Terminology Criteria for Adverse Events v4.03 (NCI CTCAE) electronically at the following Web site:

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

The Study Manual includes a copy 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
		  ○ Sodium
		  ○ Potassium
		  ○ Chloride
		  ○ 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
Biomarker Tests	• Minimal residual disease (MRD) using standardized techniques (ie, multiparametric flow cytometry [MPFC] and next generation sequencing [NGS]) and other potential biomarkers including, but not limited to, MCL-1 dependency	
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 (2017 EUROPEAN LEUKEMIANET RECOMMENDATIONS)

Category	Definition	Comment
Response		
• CR without minimal residual disease (CR_{MRD-})	If studied pre-treatment, CR with negativity for a genetic marker by real-time quantitative polymerase chain reaction (RT-qPCR), or CR with negativity by multi-color flow cytometry	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; absolute neutrophil count $\geq$1.0 x 10^9/L (1,000/µL); platelet count $\geq$100 x 10^9/L (100,000/µL)	MRD positive or unknown*
• CR with incomplete hematologic recovery (CR_i)	All CR criteria except for residual neutropenia [<1.0 x 10^9/L (1,000/µL)] or thrombocytopenia [<100 x 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

*Including patients with morphologic CR who are MRD+ and cytogenetic and/or molecular marker positive.

2017 EUROPEAN LEUKEMIANET RECOMMENDATIONS (Cont)

Category	Definition	Comment
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 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	

2017 EUROPEAN LEUKEMIANET RECOMMENDATIONS (Cont)

Category	Definition	Comment
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 months
• Progressive disease (PD)[a,b]	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 months; without at least a 100% improvement in absolute neutrophil count (ANC) to an absolute level [>0.5 x 10^9/L (500/µL), and/or platelet count to >50 x 10^9/L (50,000/µL) non-transfused]; or • >50% increase in peripheral blasts (WBC x % blasts) to >25 x 10^9/L (>25,000/µl) (in the absence of differentiation syndrome)[b]; 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 weeks 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 pre-treatment, reoccurrence of MRD as assessed by quantitative RT-qPCR or by multi-color flow cytometry	Test applied, sensitivity of the assay, and cut-off values used must be reported; analyses should be done in experienced laboratories (centralized diagnostics)

Source: Döhner H, Estey EH, Grimwade D, et al. Diagnosis and management of AML in adults: 2017 ELN recommendations from an international expert panel. 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