Sumitomo Dainippon Pharma Oncology

AMENDED CLINICAL STUDY PROTOCOL

Study Title:	A Phase 1b/2, Open-label Clinical Study to Determine Preliminary Safety and Efficacy of Alvocidib When Administered in Sequence After Decitabine or Azacitidine in Patients with MDS
Protocol Number:	TPI-ALV-102
IND Number:	057729
Study Drug/Agents:	Alvocidib (formerly flavopiridol or HMR-1275)
Phase of Development:	Phase 1b/2
Medical Monitor:	[redacted] VP, Clinical Research ***Sumitomo Dainippon Pharma Oncology, Inc*** Mobile: [redacted] Email: [redacted] Safety Reporting: [redacted]
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Date of Amendment 4:	***19 August 2020***

PROTOCOL SIGNATURE PAGE

SPONSOR SIGNATURE

I have carefully read the protocol, TPI-ALV-102 Amendment ***4,*** titled "A Phase 1b/2, Open-label Clinical Study to Determine Preliminary Safety and Efficacy of Alvocidib When Administered in Sequence After Decitabine or Azacitidine in Patients with MDS" and confirm this is the approved current version.

[redacted]

Printed Name

Date (DD/MMM/YYYY)

INVESTIGATOR'S SIGNATURE

I have carefully read this protocol, TPI-ALV-102 Amendment ***4***, 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

AE	Adverse event
ACM	Cytarabine and mitoxantrone
ALL	Acute lymphocytic leukemia
ALP	Alkaline phosphatase
ALT	Alanine aminotransferase
AML	Acute myeloid leukemia
aPTT	Activated partial thromboplastin time
AST	Aspartate aminotransferase
AUC	Area under the concentration time curve
AUC_{0-24}	AUC from 0 to 24 hours
AUC_{0--inf}	AUC from 0 to infinity
AUC_t	AUC from 0 to time t
AZA	Azacitidine
B-CLL	B-cell chronic lymphocytic leukemia
β-HCG	Beta human chorionic gonadotropin
AUC	Area under the curve
BM	Bone marrow
BSA	Body surface area
BUN	Blood urea nitrogen
CBC	Complete blood count
CDK9	Cyclin dependent kinase 9
CFR	Code of Federal Regulations
CI	Confidence interval
CIV	Continuous intravenous
CL	Clearance using noncompartmental methods
C_{max}	Maximum concentration
CR	Complete response
CRA	Clinical research associate
CRF	Case report form
CRi	Complete Response with incomplete blood count recovery
CRO	Contract Research Organization
CRR	Complete Response Rate (CR/CRi/CRmarrow/PR/HI)
CSR	Clinical Study Report
CTCAE	Common Terminology Criteria for Adverse Events
DCF	Data clarification form
DEC	Decitabine
DLT	Dose-limiting toxicity
DSMB	Data Safety Monitoring Board
ECOG	Eastern Cooperative Oncology Group
ECG	Electrocardiogram
eCRF	Electronic case report form
ESA	Erythropoietin-stimulating agent
FAB	French-American-British (classification system)
FDA	Food and Drug Administration
GCP	Good Clinical Practice
hERG	Human Ether-à-go-go-Related Gene
Hgb	Hemoglobin
HI	Hematologic Improvement

HMA	Hypomethylating agent
IC_{50}	Inhibitory concentration in 50% of test subjects
IEC	Independent Ethics Committee
IHC	Immunohistochemistry
IND	Investigational New Drug (application)
IPSS-R	Revised International Prognostic Scoring System
IRB	Institutional Review Board
ITT	Intent-to-treat
IV	Intravenous(ly)
IVI	Intravenous infusion
IWG	International Working Group
LDH	Lactate dehydrogenase
LVEF	Left ventricular ejection fraction
MCL-1	Myeloid cell leukemia 1
MDS	Myelodysplastic syndrome
MedDRA	Medical Dictionary for Regulatory Activities
MTD	Maximum tolerated dose
NCI	National Cancer Institute
NYHA	New York Heart Association
OS	Overall survival
PD	Progressive disease
PD	Pharmacodynamic
PK	Pharmacokinetic
PR	Partial response
PRN	As needed
PS	Performance status
PT	Prothrombin time
QTc	QT interval (corrected)
RBC	Red blood cell
RP2D	Recommended Phase 2 Dose
RT-aPCR	Quantitative reverse transcription-polymerase chain reaction
SAE	Serious adverse event
SAS	Statistical Analysis System (software)
SD	Stable disease
SGOT (AST)	Serum glutamic-oxaloacetic transaminase
SGPT (ALT)	Serum glutamic-pyruvic transaminase
SIV	Study initiation visit
SOP	Standard operating procedure(s)
SPRT	Sequential Probability Ratio Test
$T_{1/2}$	Half-life
TEAE	Treatment-emergent adverse event
TLS	Tumor lysis syndrome
T_{max}	Time to maximum concentration
ULN	Upper limit of normal
WHO	World Health Organization
XIAP	X-linked inhibitor of apoptosis

SYNOPSIS

Title of Study:	A Phase 1b/2, Open-label Clinical Study to Determine Preliminary Safety and Efficacy of Alvocidib When Administered in Sequence After Decitabine or Azacitidine in Patients with Myelodysplastic Syndromes (MDS)
Clinical Phase:	Phase 1b/2
Study Center(s):	Phase 1b: US: Up to approximately 15 sites Phase 2: US: Up to approximately 25 sites
Study Indication:	Myelodysplastic Syndromes
Patient Population:	• Patients with previously untreated MDS • Patients with MDS who have received <6 cycles of treatment with hypomethylating agents (HMAs) • Patients with de novo (cause unknown) or secondary MDS (treatment-related) who are not eligible for intensive induction chemotherapy or stem cell transplant o All French-American-British (FAB) subtypes (refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia) o Intermediate and above per the Revised International Prognostic Scoring System (IPSS-R) groups
Planned Enrollment and Study Duration:	Phase 1b: Approximately 12–18 months to enroll up to 24 patients in dose escalation portion up to determination of the Maximum Tolerated Dose (MTD) and Recommended Phase 2 Dose (RP2D) Expansion at MTD: Approximately 6-8 months to enroll up to 25 patients in an Expansion cohort at MTD. Data collected from these patients will be used to confirm safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity. Phase 2: Approximately 18 months to enroll up to 25 patients to confirm efficacy Total study time (Phase 1b, Expansion at MTD, and Phase 2) will be approximately 36 to 44 months.
Objectives:	Phase 1b *Primary*: • To determine the MTD and RP2D of alvocidib administered in sequence after decitabine (DEC) or azacitidine (AZA) in patients with previously untreated MDS and patients with MDS who have received <6 cycles of treatment with HMAs

	Secondary: • To determine the Complete Response Rate ([CRR]: Complete response [CR] / Complete Response with Incomplete blood count recovery [CRi] / CR in marrow / Partial Response [PR] / Hematologic Improvement [HI]) • To determine if treatment with alvocidib administered in sequence after DEC ***(during dose escalation)*** or AZA results in improvements in transfusion dependence (defined as an average transfusion requirement of ≥2 units/28 days of RBCs without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose) and/or hemoglobin level (transfusion data to include the hemoglobin value for which the transfusion was administered and must have been ≤10.0 g/dL within 7 days prior to transfusion administration to be counted toward the RBC transfusion-dependent status): o Hemoglobin increase of ≥1.5 g/dL without erythropoietin-stimulating agents (ESAs) *OR* o Reduction of ≥4 RBC transfusions/8 weeks in comparison to pre-treatment values and lasting at least 8 weeks *Exploratory*: • To evaluate MCL-1 dependence in untreated MDS patients via BH3 profiling • To determine whether DEC ***(during dose escalation)*** or AZA treatment modulates MCL-1 dependence in peripheral blood <u>Phase 2</u> *Primary*: • To determine preliminary efficacy and anti-MDS activity of alvocidib administered in sequence after AZA in untreated patients with de novo or secondary MDS *Secondary*: • To assess the CRR per the revised International Working Group (IWG) Criteria • To evaluate the tolerability of alvocidib when administered in sequence following AZA in this patient population • To determine whether a defined BH3 profile predicts response to alvocidib administered in sequence after AZA in untreated MDS population • To determine if treatment with alvocidib administered in sequence after AZA results in improvements in transfusion dependence (defined as an average transfusion requirement of ≥2 units/28 days of RBCs without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately

	preceding first dose) and/or hemoglobin level (transfusion data to include the hemoglobin value for which the transfusion was administered and must have been ≤10.0 g/dL within 7 days prior to transfusion administration to be counted toward the RBC transfusion-dependent status): ○ Hemoglobin increase of ≥1.5 g/dL without ESAs *OR* ○ Reduction of ≥4 RBC transfusions/8 weeks in comparison to pre-treatment values and lasting at least 8 weeks *Exploratory Objectives:* • To assess the pharmacokinetics (PK) of alvocidib when administered in sequence after AZA • To evaluate correlative biomarkers of therapy including, but not limited to, BH3 profiling with an emphasis on MCL-1 dependence, genetic mutations, and other biomarkers associated with MDS • To document the percentage of patients transplanted post-treatment and outcomes post-transplant Additional exploratory analyses may be performed if useful in the interpretation of the data.
Study Design:	Phase 1b: 3+3 dose-escalation design Expansion at MTD: using MTD determined in Phase 1b study, up to 25 patients will be enrolled in an Expansion cohort to receive alvocidib following AZA to confirm safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity Phase 2: using optimal dose from the Phase 1b study, initiate fixed-dose study of approximately 25 patients in Simon 2-stage minimax design.
Inclusion Criteria:	To be eligible for participation in the study, patients must meet all of the following inclusion criteria: 1. Aged ≥18 years 2. Phase 1b ***Dose Escalation***: Patients with previously untreated MDS and patients with MDS who received fewer than six (6) cycles of previous HMAs ***Phase 1b Expansion: Untreated patients with de novo or secondary MDS*** Phase 2: Untreated patients with de novo or secondary MDS 3. Patients with an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) score ≤2 at enrollment 4. 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.) 5. Patients with a life expectancy of ≥3 months (90 days) 6. Patients with adequate major organ functions meeting the following criteria on the basis of laboratory data within 4 weeks (28 days) before enrollment (if multiple data are available, most recent data during the period): a. Serum creatinine: ≤1.8× the upper limit of the normal (ULN) range b. Total bilirubin: ≤2× the ULN c. Aspartate transaminase (AST) and alanine transaminase (ALT): ≤3× the ULN 7. Be able to comply with the requirements of the entire study. 8. ***Patients with Revised International Prognostic Scoring System (IPSS-R) intermediate-, high-, and very high-risk MDS***
Exclusion Criteria:	Patients meeting any one of these exclusion criteria will be prohibited from participating in the study. 1. Presence of concomitant severe cardiovascular disease: a. Patients who had myocardial infarction within 6 months (180 days) before enrollment b. Patients with significant diseases at enrollment that may affect study treatment, such as New York Heart Association (NYHA) Functional Class III or IV heart disease, National Cancer Institute (NCI) Common Terminology Criteria for Adverse Events (CTCAE) v5.0 grade ≥3 arrhythmia, angina pectoris, abnormal electrocardiogram findings, interstitial pneumonia or pulmonary fibrosis 2. Presence of concomitant malignancy requiring chemotherapy or any malignancy (except basal and squamous cell carcinoma of the skin) for which the patient received chemotherapy within 6 months prior to enrollment. NOTE: Diagnosis of any previous or concomitant malignancy is thus not an exclusion criterion. 3. Presence of uncontrolled or uncontrollable infection(s); or ≥Grade 3 infection according to NCI CTCAE v5.0 4. Presence of any psychological, familial, sociological, or geographical condition that, in the opinion of the investigator, could potentially hinder compliance with the study protocol and follow-up schedule 5. Patients with a dry tap on bone marrow aspiration before enrollment

	6. Patients with concurrent autoimmune disease or a history of chronic or recurrent autoimmune disease, or patients who require long-term systemic steroid therapy greater than the equivalent of 20 mg of prednisone daily (excluding therapy given on an 'as needed' [PRN] basis) 7. Patients with other documented malignancies within past year aside from synchronous or metachronous multiple cancers with a disease-free period of ≤5 years (excluding carcinoma in situ, mucosal carcinoma, or other such carcinomas curatively treated with local therapy) 8. Patients with ≥Grade 2 hemorrhage according to NCI CTCAE v5.0 9. Patients who have previously received alvocidib or another cyclin-dependent kinase 9 (CDK9) inhibitor 10. Patients who are pregnant or breastfeeding 11. Female patients of childbearing potential who are sexually active and unwilling to use a medically acceptable method of contraception associated with a low failure rate during and for at least 6 months after the last dose of study drug (Patients will be considered to be of childbearing potential unless surgically sterilized by hysterectomy, or bilateral tubal ligation / salpingectomy, or postmenopausal for at least 2 years.) 12. Male patients with partners of childbearing potential who are unwilling to use condoms in combination with a second effective method of contraception during the trial and for at least 6 months after the last administration of study treatment. 13. Patients who are inappropriate for participation in the study for other reasons in the opinion of the investigator or sub-investigator(s) 14. Patients with a known hypersensitivity to DEC ***(those patients enrolled in dose escalation)***, AZA***,*** or mannitol 15. ***Patients who have received erythropoietin-stimulating agents (ESAs) within 2 weeks (14 days) prior to Cycle 1/Day 1***
Study Treatment:	Phase 1b Patients will receive alvocidib administered in sequence after DEC ***(during dose escalation)*** or AZA. Decitabine is administered as a 1-hour intravenous (IV) infusion (IVI) daily for 5 days at a dose of 20 mg/m^2 followed on Day 8 by alvocidib as a loading dose over 30-minutes followed by a 4-hour IVI according to the following schedule.

Dose Level [a]	Days 1-5, Decitabine	Day 8, Alvocidib [b]	
	1-hr IV infusion	30-min bolus	4-hr IV infusion
1	**20 mg/m^2**	**20 mg/m^2**	**20 mg/m^2**
2	20 mg/m^2	30 mg/m^2	30 mg/m^2
3	20 mg/m^2	30 mg/m^2	45 mg/m^2
4	20 mg/m^2	30 mg/m^2	60 mg/m^2

a It is possible for additional and/or intermediate dose levels to be added during the course of the study.
b Alvocidib to be administered first as a 30-minute (±10 minutes) IV bolus followed up to 30 minutes later by a 4-hour (±15 minutes) IVI.

Once the MTD of alvocidib administered via hybrid dosing (ie, 30-minute bolus followed by a 4-hour IVI) has been determined, 2 cohorts of patients (minimum of 6 patients; 3 per cohort) will receive AZA followed by alvocidib administered as a ***30- to 60-minute*** IVI. The dose of alvocidib in the first AZA cohort will be 75 mg/m^2. In the absence of any significant toxicities, the alvocidib dose will be escalated.

Azacitidine may be administered on either a 7-day schedule (ie, 7 consecutive days) or a 5-2-2 schedule (ie, once daily for 5 days followed by 2 drug-free days with 2 more days of treatment. Azacitidine may be given as an IVI over 10 to 40 minutes or as a subcutaneous (SC) injection. Regardless of which AZA schedule or route of administration is used, alvocidib will be given on Day 10 as a ***30- to 60-minute*** IVI. Choice of schedule and route of administration of AZA will be at the discretion of the investigator.

Dose Level [a]	Azacitidine [b]	Day 10, Alvocidib
	IVI or SC injection [c]	***30- to 60-minute*** IVI
1A	75 mg/m^2	75 mg/m^2
1B	75 mg/m^2	90 mg/m^2

a It is possible for additional and/or intermediate dose levels to be added during the course of the study.
b AZA can be administered on either a 7-day or 5-2-2 schedule.
c AZA may be given as an IVI over 10 to 40 minutes or an SC injection.

Expansion at MTD

Using the MTD of alvocidib administered as a ***30- to 60-minute*** IVI determined during Phase 1b, up to 25 patients will be enrolled in an Expansion cohort to receive alvocidib following AZA to confirm safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity.

Phase 2

The Phase 2 study will use the RP2D of alvocidib administered by ***30- to 60-minute*** IVI from the Phase 1b study and follow a Simon 2-stage minimax design.

All patients are eligible to receive a minimum of 4 cycles of treatment.

	Additional cycles may be considered following discussions between the investigators and the Medical Monitor.
	
	Supportive care measures for all patients to include:
	
	• Infection prevention (antibiotics, antifungals, antivirals) according to institutional standards
	• Routine growth factor support is not allowed. Growth factor support can be given at the discretion of the Investigator and with the Medical Monitor's approval in the presence of life threatening infection with ongoing neutropenia.
	
	<u>TLS Prophylaxis (DEC + ALV)</u>
	
	• All patients receiving DEC ***(during dose escalation)*** will receive TLS prophylaxis as per each institution's standard of care
	
	<u>TLS Prophylaxis (AZA + ALV)</u>
	
	All patients receiving alvocidib in sequence following AZA will receive TLS prophylaxis as detailed below:
	
	• IV Hydration
	  o Prior to AZA
	  ▪ IV hydration according to each institution's standard of care
	  o Prior to Alvocidib
	  ▪ Mandatory IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 500 cc for 1-2 hours prior to alvocidib, then an additional 500 cc for 1-2 hours after alvocidib during Cycle 1 (volume may be reduced to between 250 cc-500 cc, if clinically indicated). Hydration is optional for subsequent cycles.
	• Diarrhea Prophylaxis
	  o During AZA and Alvocidib Dosing
	  ▪ Replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated.
	  o Alvocidib-specific Measures
	  ▪ Alvocidib can induce diarrhea when given over a short period of time. Over-the-counter measures are typically effective in this setting if initiated early.
	  ▪ It is strongly suggested that patients take 2 tablets of loperamide, 2 mg each (or equivalent), prior to the alvocidib IVI and then take 1 tablet (2 mg) for every loose stool up to a maximum of 8 tablets (16 mg) in a 24-hour period.
	  ▪ Persistent diarrhea despite optimal outpatient management would trigger medical consultation. Early consideration should be given for possible *Clostridioides*

	difficile (*C. difficile*) infection in this patient population and identifying/treating as expeditiously as possible should be top of mind. • Oral Allopurinol o Mandatory oral allopurinol to be started on Day 1 of Cycle 1 and continued daily for the first 2 weeks (ie, 14 days). This may be discontinued for subsequent treatment cycles if uric acid levels are within normal limits and there is no evidence of tumor lysis syndrome. • Oral Phosphate Binder o Mandatory oral phosphate binder to be started on Day 1 of Cycle 1 and continued daily for the first 2 weeks (ie, 14 days). o If serum phosphorus levels are <3 after the first treatment with alvocidib and there is no evidence of TLS, phosphate binders may be discontinued. Patients should continue to be monitored for TLS as outlined for subsequent treatment cycles. Caution is warranted for patients who still have a high blast count as they remain at risk for TLS with subsequent treatments. • Laboratory Evaluations for Tumor Lysis Syndrome ('tumor lysis labs'): o 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 During Cycle 1 ▪ Monitor tumor lysis labs prior to first AZA and 2 hours (±30 minutes) after completion of first AZA dose ▪ Monitor tumor lysis labs prior to alvocidib IVI and 2 hours (±30 minutes) after completion of IV hydration post alvocidib. ▪ All tumor lysis labs should be drawn; however, the potassium level obtained at 2 hours post first AZA dose and 2 hours post hydration following alvocidib IVI should be reviewed immediately to determine if additional treatment is warranted (see *Section 4.5.1.2*). ▪ Labs will also be drawn daily for the first 2 days following first AZA dose (ie, Days 2-3) and alvocidib (ie, Days 11-12) and at least weekly for the remainder of Cycle 1. o During Cycle 2, tumor lysis labs will be assessed: ▪ Prior to C2D1 first AZA dose and at 2 hours post C2D1 AZA dose

	▪ Prior to C2D10 alvocidib IVI and 2 hours (±30 minutes) after completion of alvocidib IVI. o During Cycles 3+, tumor lysis labs will be assessed at the discretion of the investigator in relation to patient blast counts 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.
Treatment Plan:	Phase 1b Patients will be enrolled in cohorts of 3-6 patients. Escalation of the alvocidib dose will follow a standard 3+3 design with sequential cohorts of 3 patients treated with incrementally higher doses of alvocidib until a dose-limiting toxicity (DLT) is observed and the MTD is established. The first 2 patients at a dose level may be enrolled simultaneously and third patient will be enrolled after 14 days so long as the 2 initial patients have not experienced any unacceptable toxicity. *Dose-limiting toxicities* will be defined as: • Any Grade 5 toxicity that is not clearly and incontrovertibly related to the underlying disease or extraneous causes • Any Grade 4 nonhematologic toxicity considered at least possibly drug related • Grade 4 neutropenia lasting ≥42 days from the start of a cycle in the absence of evidence of active disease • Any AST or ALT elevation ≥3x ULN accompanied by serum bilirubin levels >2x ULN • 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 bilirubin, AST, ALT or alkaline phosphatase will be considered dose-limiting only if resolution to ≤Grade 2 requires more than 7 days 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 o ≥Grade 3 creatinine elevation that does not resolve to ≤Grade 2 within 7 days o Anorexia, fever, neutropenic fever, and infections of any grade • Bone marrow hypoplasia that occurs for >42 days with bone marrow (BM) cellularity ≤5% and no evidence of MDS/leukemia. Bone marrow disease assessments conducted per protocol on

	Day 28 (±3 days) of every even cycle may be used to guide this decision. If 1 of 3 patients in a cohort experiences a DLT, up to 3 additional patients will be treated at that dose level. If no additional DLTs are observed in the expanded 3- to 6-patient cohort within 28 days after the last patient was first dosed, the 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. Adverse events meeting the definition of DLT during Cycles 2+ will be taken into consideration when evaluating dose escalation. Once the MTD of ALV has been determined using the hybrid dosing schedule (ie, 30-minute IV bolus + 4-hour IVI), 2 cohorts of at least 3 patients each will receive AZA followed in sequence by alvocidib administered as a ***30- to 60-minute*** IVI. <u>Expansion at MTD</u> Once the MTD or preliminary RP2D of alvocidib administered by ***30- to 60-minute*** IVI is identified, up to 25 patients will be enrolled in an Expansion cohort to receive alvocidib following AZA to confirm safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity. Once this Expansion cohort is completed, the study will progress to Phase 2. <u>Phase 2</u> *Stopping Rules based on Efficacy* The Phase 2 design is based on the Simon 2-stage minimax design (Simon 1989). • Stage 1: Up to 15 evaluable patients will be enrolled and treated at the RP2D identified in the Phase 1b study. Stage 2 may be initiated at any point after confirming a response (CR/CRi/CRmarrow/PR/HI) in two Stage 1 patients. If there is ≤1 responder among 15 evaluable Stage-1 patients, the study will be stopped after Stage 1. • Stage 2: Ten patients will be enrolled to bring the total enrollment in Phase 2 (including Stage-1 patients) to 25 evaluable patients. Stage-2 patients will also receive the RP2D dose of alvocidib administered by ***30- to 60-minute*** IVI identified in the Phase 1b study. If 6 or more responses are observed in 25 patients, the conclusion will be that the combination regimen is worthy of further investigation. When the true response rate of 30% (alternative hypothesis) is tested against the null hypothesis

	response rate of 10%; this design yields a Type I error rate of 0.05 and power of 80%. Any patient who withdraws from Stage 1 or 2 for treatment-related toxicity or disease progression, or dies prior to being evaluated for response will be considered a nonresponder. Patients who drop out for other reasons prior to being assessed for response will be considered unevaluable and may be replaced. Enrollment into Phase 2 may be stopped at any point once ≥6 patients have had a response to treatment, but the maximum enrollment in Phase 2 will be 25 evaluable patients. *Stopping Rules based on Safety* Early stopping rules for safety are based on a Sequential Probability Ratio Test (SPRT) with a baseline toxicity rate of 5%; an upper ceiling of unacceptable toxicity of 20% (ie, toxicities meeting one or more DLT criteria as stated in *Section 4.5.3*); and alpha=0.05 with power=80%. The study will be stopped if unacceptable toxicities are observed in: • 2 of the first 2 patients • 3 of the first 11 patients • 4 of the first 20 patients • 5 of the first 29 patients (Ph 2 study to enroll maximum of 25 patients, but considering possible replacement patients)

Study Assessments:	These assessments apply to both phases of the study.
	
	<u>Predose</u>
	
	• *Screening Period (Within 28 Days Prior to First Dose of Cycle 1)*
	  ○ Informed Consent
	  ○ Complete medical and disease history
	  ○ Complete physical examination (all body systems) and including height (cm) and weight (kg)
	  ○ Vital signs (body temperature, heart rate, systolic and diastolic blood pressures)
	  ○ ECOG performance status
	  ○ Hematology panel: complete blood cell count (CBC) with manual differential and platelet count
	  ▪ ***Record all transfusions (hemoglobin and platelet) for the 8 weeks/56 days prior to screening (including the transfusion date and number of units with each transfusion)***
	  ▪ ***Record all CBC data for the 8 weeks/56 days prior to screening including each CBC that prompted a transfusion***
	  ○ Full serum chemistry panel
	  ○ Coagulation parameters: prothrombin time (PT) and activated partial thromboplastin time (aPTT)

	○ Serum or urine pregnancy test in females of childbearing potential ○ Perform a 12-lead electrocardiogram (ECG) including assessment of corrected QT interval (QTc) ○ Chest radiograph ('x-ray') (if not done within previous 28 days) ○ Perform bone marrow biopsy and/or aspiration and collect peripheral blood for disease status, standard cytogenetics, and pharmacodynamic (PD) analyses. If the initial bone marrow aspirate is nonproductive or not diagnostic, the procedure must be repeated. ○ Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements within the previous 28 days • *Baseline Period (Within 72 Hour Prior to First Dose of Cycle 1)* ○ Complete physical examination including weight (kg) and calculation of body surface area (BSA) ○ Vital signs ○ ECOG performance status ○ Hematology panel ○ Full serum chemistry panel ○ Serum or urine pregnancy test in females of childbearing potential ○ Administer prophylactic antibiotics, antivirals, antifungals, antiemetics, antidiarrheals according to standard practices at each investigational site (Day 1) ○ Administer TLS prophylaxis prior to first DEC dose according to standard practices at each investigational site ○ Administer TLS prophylaxis prior to first AZA and alvocidib doses per the guidelines provided in *Section 4.5.1.1.2* ○ Concomitant medications taken since previous predose assessment visit <u>Treatment Period</u> • Administer TLS prophylaxis prior to first DEC dose ***(during dose escalation)*** according to standard practices at each investigational site • Administer TLS prophylaxis prior to first AZA and alvocidib doses ○ AZA-specific TLS prophylaxis ▪ Administer pretreatment IV hydration per institutional standards ▪ Begin oral allopurinol daily from Days 1-14 of Cycle 1 ▪ Begin oral phosphate binder treatment daily from Days 1-14 of Cycle 1

	▪ Replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated (see *Section 4.5.1.2* and *Section 4.5.1.3*) ○ Alvocidib-specific TLS prophylaxis ▪ Administer pretreatment IV hydration at least 2 hours prior to first dose of alvocidib ▪ Continue oral allopurinol daily from Days 1-14 of Cycle 1 ▪ Continue oral phosphate binder treatment daily from Days 1-14 of Cycle 1 (see *Section 4.5.1.1.2*) ▪ Replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated (*Section 4.5.1.2* and *Section 4.5.1.3*) • Just Prior to First Dose of DEC, AZA, and Alvocidib ○ Record vital signs measured 5-15 minutes prior to the initiation of infusion following a 5-minute rest ○ Collect blood for evaluation of laboratory parameters (*Appendix E*): ▪ Tumor lysis labs ○ 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. • At 2 hours post initial AZA dose or post IV hydration (alvocidib), draw tumor lysis labs and immediately assess potassium level for indications of tumor lysis • Each Cycle: Weekly Unless Otherwise Stated ○ Abbreviated physical examination (AE- or symptom-directed exam) ○ Vital signs ○ ECOG performance status ○ CBC with differential and platelet count ○ Full serum chemistry panel ○ Response assessments and standard cytogenetics at end of Cycles 2, 4, 6, and then every 4 cycles, thereafter, or as clinically indicated ○ Assessment of BH3 profiling and other potential biomarkers in peripheral blood (as indicated) and bone marrow aspirates (whenever samples are collected for response assessments) ○ Assessment of AEs ○ Concomitant medications associated with an AE ○ ***Record all transfusions (hemoglobin and platelet) including the transfusion date and number of units with each transfusion***

Pharmaco-kinetic Assessments:	PK parameters assessed using noncompartmental methods to include: • C_{max} = maximum observed plasma concentration • T_{max} = time to C_{max} (peak time) • AUC_{0-24} = area under the plasma concentration curve from time 0 to 24 hours • AUC_{0-inf} = AUC from time 0 to infinity • AUC_t = area under the plasma concentration curve from time 0 to time t • $t_{1/2}$ = half life • CL = clearance using noncompartmental methods
Pharmaco-dynamic Assessments:	Peripheral blood and bone marrow samples will be collected at protocol-specific time points to assess the effects of alvocidib when administered in sequence after DEC ***(during dose escalation)*** or AZA. Analyses may include, but are not limited to, assessment of BH3 profiling by flow cytometry with an emphasis on MCL-1 dependence, evaluating genetic mutations, and other biomarkers associated with MDS. The samples may be retained for no longer than 20 years after study completion or per local requirements.
Safety Endpoints:	<u>Phase 1b</u> Safety and tolerability of alvocidib when administered in sequence after DEC ***(during dose escalation)*** or AZA will be assessed by analyzing DLTs, MTD, and incidence rates of treatment-emergent adverse events (TEAEs) summarized within treatment group(s) 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). Adverse events will be graded according to NCI CTCAE v5.0. Other routine safety assessments (eg, physical examinations, vital sign measurements, and clinical laboratory testing (hematology and chemistry) will be evaluated as measures of safety and tolerability for the entire study duration. These assessments will be summarized by shift tables and treatment group using mean, standard deviation, median, minimum and maximum changes from baseline values. Mortality (all causes) will be calculated. <u>Phase 2</u> During the Phase 2 study, tolerance and toxicity will be assessed through evaluation of physical examinations, vital signs, laboratory studies, solicited and unsolicited adverse events, and all causes of mortality at 30 and 60 days. Routine safety assessments will be summarized by shift tables using mean, standard deviation, median, minimum and maximum changes from baseline values. Mortality (all causes) will be calculated.

Efficacy Endpoints:	Phase 2 *Primary Efficacy Endpoints* • Assess preliminary efficacy, as determined by response rate, duration of response, hematological improvement, rate of transfusion independence, time to acute myeloid leukemia (AML), and overall survival (OS). *Efficacy endpoints will be any objective response to study drug therapy using assessments defined by the 2006 revised International Working Group (IWG) and European MDS Working Group.* *Secondary Efficacy Endpoints* • The proportion of MDS patients in the overall population achieving RBC transfusion independence with duration ≥84 days (12 weeks) during treatment. Baseline RBC transfusion dependency is defined as an average transfusion requirement of ≥2 units/28 days of RBCs without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose. Transfusion data include the hemoglobin value for which the transfusion was administered and must have been ≤10.0 g/dL within 7 days prior to transfusion administration to be counted toward the RBC transfusion-dependent status. • Platelet transfusion independence with duration ≥84 days (12 weeks) will be assessed in the proportion of patients who were platelet transfusion-dependent at baseline. Baseline platelet transfusion requirements are established by the number of platelet transfusions administered during the 56 days immediately preceding and including the date of first dose. • Erythroid hematologic improvement and platelet hematologic improvement will be assessed according to the 2006 revised IWG criteria. • Correlation of complete response rates with BH3 profiling results including MCL-1 dependency • Overall survival compared to historical survival *Complete details of the planned analysis will be documented in a full Statistical Analysis Plan (SAP), which will be finalized before locking the study database*.
PK Endpoints:	Plasma concentrations of alvocidib will be summarized by descriptive statistics, including mean, n, standard deviation, coefficient of variation, minimum, maximum, and median. Prior to analysis of study samples, the assay sensitivity, specificity, linearity, and reproducibility will be documented. Plasma PK analyses for alvocidib and known metabolites, if any, and dose proportionality will be determined on Days 8 and 9 (for patients receiving alvocidib following DEC ***during dose escalation***) or Days 10 and 11 (for patients receiving alvocidib following AZA) of

Cycle 1 in all patients enrolled in the Phase 1b study.

Cycle 1, Day 8 (DEC+ALV) / Cycle 1, Day 10 (AZA+ALV):

PK Sample No.	Timepoint in Relation to Alvocidib Administration	Window
1	Prior to IVI	At any time
2	End of IVI	±10 minutes
	Up to 30-minute wait (±10 minutes) [a]	
3	30 mins after end of IVI	±10 minutes
4	1 hr after end of IVI	+10 minutes
5	2 hours after end of IVI	±15 minutes
6	4 hrs after end of IVI	±15 minutes

a This wait time is only required in patients receiving DEC followed by alvocidib hybrid dosing (ie, 30-min IV bolus; up to 30-min wait; and then the 4-hr IVI).

Cycle 1, Day 9 (DEC+ALV) / Cycle 1, Day 11 (AZA+ALV):

PK Sample No.	Timepoint in Relation to Alvocidib Administration	Window
7	After alvocidib IVI (23 hrs after start of 4-hr IVI in pts receiving hybrid dosing or ***start*** of infusion in pts receiving ***30- to 60-min*** IVI)	±1 hour

PD Endpoints:

- Determine any possible correlation between the rate of CR/CRi/CRmarrow/PR/HI and BH3 profiling by flow cytometry with an emphasis on MCL-1 dependence.
- The types of biomarkers to be analyzed may include, but are not limited to, nucleic acids, proteins, lipids or metabolites. Biomarker assessments may be used to assess and generate prognostic, predictive, or surrogate biomarker signatures. These assessments may be explored in the context of MDS or related conditions or drugs of similar class. The results from these analyses are exploratory in nature and may not be included in a clinical study report (CSR).

DEC + ALV PD Sample Collection Schedule

PD Sample No.	Visit/Day	Timepoint in Relation to Drug Administration	Window
1	Screening	Day -28 to Day 1	At any time
2	C1D1	Prior to DEC infusion	≤10 mins prior
3	C1D3	Prior to DEC infusion	≤10 mins prior
4	C1D5	Prior to DEC infusion	≤10 mins prior
5	C1D8	Prior to alvocidib IVI	At any time
6	C1D9	After alvocidib IVI (23 hrs after start of 4-hr IVI)	Within ±1 hr
7	C1D15	During Study Visit	±3 days
8	C1D22	During Study Visit	±3 days
9	C2D1	Prior to DEC infusion	At any time
10	C2D8	Prior to alvocidib IVI	At any time
11	C2D15	During Study Visit	±3 days

	12	C2D28	During Study Visit	+1/-3 days
	13	C4* + D28	During Study Visit	At any time
	14	EOS	During Study Visit	At any time

*Even cycles 4 ***and 6 and every 4 cycles thereafter (ie, Cycle 10, Cycle 14, etc).***

AZA + ALV PD Sample Collection Schedule

PD Sample No.	Visit/Day	Timepoint in Relation to Drug Administration	Window
1	Screening	Day -28 to Day 1	At any time
2	C1D1	Prior to AZA infusion	≤10 mins prior
3	C1D3	Prior to AZA infusion	≤10 mins prior
4	C1D5	Prior to AZA infusion	≤10 mins prior
5	C1D7	Prior to AZA infusion	≤10 mins prior
6	C1D10	Prior to alvocidib IVI	At any time
7	C1D11	After alvocidib IVI (23 hrs after ***start*** of infusion)	Within ±1 hr
8	C1D15	During Study Visit	±3 days
9	C1D22	During Study Visit	±3 days
10	C2D1	Prior to AZA infusion	At any time
11	C2D10	Prior to alvocidib IVI	At any time
12	C2D15	During Study Visit	±3 days
13	C2D28	During Study Visit	+1 / -3 days
14	C4* + D28	During Study Visit	At any time
15	EOS	During Study Visit	At any time

*Even cycles 4 ***and 6 and every 4 cycles thereafter (ie, Cycle 10, Cycle 14, etc).***

Additional exploratory analyses may be performed if useful in the interpretation of the data and/or to assist the sponsor in planning future studies.

1. INTRODUCTION

1.1 BACKGROUND

Myelodysplastic syndromes (MDS) are a heterogeneous group of hematopoietic stem cell disorders. They are characterized by dysplasia in the myeloid, megakaryocytic and/or erythroid lineages. The abnormal cells belong to a malignant clone, which represses the remaining normal cells in the bone marrow. Patients suffer from peripheral blood cytopenias (anemia, leukopenia and/or thrombocytopenia).

The overall incidence of MDS is estimated to be 3 to 4 cases per 100,000 persons per year. Greater than 85% of these patients are older than 60 years at the time of the diagnosis. It is generally assumed that the incidence is underestimated due to the complexity of diagnosing MDS. Indolent forms of MDS, when blast counts are normal or only slightly elevated are difficult to diagnose. However, there are a number of other conditions, such as infections or medication that can result in transient cytopenias and dysplastic cells, without clonal aberrations. In approximately 50% of patients, chromosomal abnormalities are found using conventional cytogenetics, which can facilitate the diagnosis of MDS.

The natural course of MDS ranges from an indolent disease without impaired survival that can be controlled with supportive care, to a more acute manifestation with severe bone marrow failure resulting in life-threatening complications. About 30% of the patients diagnosed with MDS progress to develop acute myeloid leukemia (AML). The primary cause of mortality in MDS patients is from complications of bone marrow failure.

1.2 RATIONALE

Treatment options for patients with MDS include supportive care, high intensity and/or low intensity drug therapy and, in a small proportion of patients, stem cell transplantation.

Supportive care is typically targeted at addressing anemia as well as infections or other comorbidities related to ineffective bone marrow function. Supportive care may include transfusion therapy, erythropoiesis-stimulating agents and antibiotic therapy.

Drug therapy includes:

- Lenalidomide for those with myelodysplastic syndrome associated with an isolated del(5q) chromosome abnormality who need frequent red blood cell transfusions
- Immunosuppressive therapy to decrease erythrocyte turnover and therefore lessen the need for red blood cell transfusions.

- Hypomethylating agents (HMAs), ie, azacitidine (AZA) and decitabine (DEC)
- Chemotherapy with stem cell transplant

Alvocidib in time-sequential therapy demonstrated significant clinical activity in secondary AML patients with prior MDS. Alvocidib, a cyclin-dependent kinase 9 (CDK 9) inhibitor is being developed for the treatment of AML; is involved in regulating of transcription through activation of RNA polymerase 2. In cancer biology, CDK 9 is particularly relevant as a key enzyme regulating levels of myeloid cell leukemia (MCL-1), an important survival factor that is controlled by RNA polymerase. Alvocidib has been evaluated in patients with solid tumors and hematologic malignancies. Eight Phase I or II clinical trials have been completed in patients with AML, totaling more than 400 patients with both relapsed/refractory or newly diagnosed AML. In these trials, alvocidib was evaluated as a single agent as well as in time-sequential therapy with cytarabine and mitoxantrone (ACM).

Patients with IPSS-R intermediate and above MDS have an increased risk of developing AML and may be treated with the same chemotherapy regimens used in patients with AML.

Preclinical studies have demonstrated that decitabine exposure increased the expression of NOXA, which is a specific antagonist of the survival factor MCL-1. Pharmacologic downregulation of MCL-1 via CDK 9 inhibition, as well as upregulation of the MCL-1 antagonist, NOXA, following decitabine exposure may result in enhanced antileukemic activity in MCL-1-dependent malignancies. In addition, MV411 cells pretreated with alvocidib were shown to be more sensitive to subsequent treatment with another HMA, azacitidine.

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.

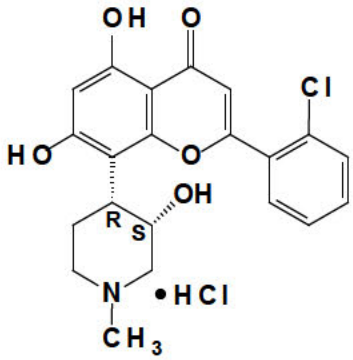
2.1 BACKGROUND

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 MECHANISM OF ACTION

Alvocidib is a potent cyclin-dependent kinase (CDK) inhibitor with selectivity for CDKs 9, 1, 2, 4 and 7 [1, 2, 3]. The greatest inhibition (K_i of 3 nM) was observed with CDK 9 [4]. Alvocidib-induced apoptosis is driven 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 [5, 6, 7]. 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 [8, 9]. 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 [10].

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 [11].

Patients with IPSS-R intermediate and above MDS have an increased risk of developing AML and may be treated with the same chemotherapy regimens used in patients with AML.

Preclinical studies have demonstrated that decitabine exposure increased the expression of NOXA, which is a specific antagonist of the survival factor MCL-1. Pharmacologic downregulation of MCL-1 via CDK 9 inhibition, as well as upregulation of the MCL-1 antagonist, NOXA, following decitabine exposure may result in enhanced antileukemic activity in MCL-1 dependent malignancies.

2.4 PRECLINICAL STUDIES

2.4.1 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 [1, 2, 3]. 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) [4]. 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 [12].

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 [13, 14]. 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) [5, 7]. 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 Non-Hodgkin'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 [15]. 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 demonstrated only minor activity when evaluated in vivo in various solid tumor models.

2.4.2 Safety Pharmacology

Alvocidib produced no significant effects on the central nervous, respiratory, or hematological systems, however, alvocidib at 1 µM produced a slightly increased contractile force (+24%) and a decreased rate of contractions (-26%) in isolated guinea pig atria. Alvocidib slightly blocked in vitro Human Ether-à-go-go-Related Gene (hERG) currents, with an average IC_{50} value of 3.2 µmol/L (1286 ng/mL). Alvocidib produced no physiologically relevant modifications of hemodynamic parameters or electrocardiogram in anesthetized dogs when given intravenously at 0.8 mg/kg (16 mg/m^2) during a 1-hour infusion.

2.4.3 Nonclinical Absorption, Distribution, Metabolism and Excretion Studies

Nonclinical pharmacokinetic studies in intravenously treated mice, rats, and dogs showed that plasma clearance was high (3.5 L/h/kg in rats and 1.4 L/h/kg in dogs) and elimination half-lives ($t_{1/2}$) were short (1.9 hours in rats and 1.1 hours in dogs). In vitro protein binding of the drug was concentration independent. Binding was highest in human plasma (>90%) and slightly lower in rat (~88%) and dog (~80%) plasma. In rats, alvocidib and/or its metabolites were distributed throughout the body within 5 minutes and disappeared from organs and tissues within 72 hours.

In pigmented rats, binding of alvocidib and/or its metabolites was associated with the melaniferous tissue of the eye.

2.4.4 Animal Toxicology

Single-dose intravenous (IV) toxicity studies were conducted in rats (30-minute infusion) and dogs (30-minute or 24-hour infusion). The maximum tolerated dose (MTD) in the rat was 10 mg/kg (60 mg/m^2), whereas in the dog (30-minute infusion), the MTD was 0.75 mg/kg (15 mg/m^2). Clinical observations included vomiting (dogs), diarrhea (rats and dogs), and gastrointestinal bleeding (dogs). Deaths were attributed to gastrointestinal toxicity. Administration of alvocidib at 2.6 mg/kg (52 mg/m^2) by 24-hour continuous intravenous (CIV) infusion was nonlethal in the dog, whereas 1.5 mg/kg (30 mg/m^2) was lethal when administered as a 30-minute IV infusion.

Repeated-dose toxicity studies were conducted in rats and dogs by multiple 30-minute IV infusions and multiple 72-hour infusions. Four-week studies with daily 30-minute infusions caused mortality in rats at 3 mg/kg/day (18 mg/m^2/day) and in dogs at 0.5 mg/kg/day (10 mg/m^2/day). The dose-limiting toxicity (DLT) related to the exaggerated pharmacological properties of the compound occurred in the gastrointestinal tract, in the bone marrow, and in the lymphoid tissues. Liver enzymes were elevated in some of the studies. Toxicity was generally dose proportional and independent of gender. Most effects were partially or completely reversible within 4 weeks. In general, target organ toxicity seen with continuous infusion was comparable to that produced by daily dosing. Multiple-cycle toxicity studies (72-hour IV infusions every 2 weeks) in rats (over 3 and 6-month periods) and dogs (over a 6-month period) were associated with thrombosis, necrosis, inflammation, and fibrosis at the infusion site (vena cava) in many animals. This local toxicity was not predicted by single-dose local tolerance studies in dogs and rabbits conducted by IV, perivenous, intra-arterial, and intramuscular administration.

2.4.5 Genotoxicity

Alvocidib was found to be negative in the bacterial reverse mutation test (Ames test) (Study 98.0059, Study C350.501017) and in the hypoxanthine-guanine phosphoribosyltransferase gene mutation tests in Chinese hamster V79 cells (Study 98.0057, Study 98.0058). It was found to be positive in the mouse lymphoma assay only in the presence of metabolic activation (study C350.703). These results suggested that alvocidib could lack mutagenic activity. Alvocidib was found to be positive in the in vivo mouse bone marrow micronucleus test (study 98.0056), but negative in the in vitro chromosome aberration test in Chinese hamster V79 cells under the experimental conditions of the test (study 98.0058), suggesting that alvocidib was able to induce chromosome damage in vivo. Based on this battery of genetic toxicity studies, alvocidib was considered to be potentially genotoxic.

2.4.6 Reproductive and Developmental Toxicity

Alvocidib did not affect fertility and reproduction in mice or rats, although degeneration/necrosis of seminiferous tubular germ cells was observed in mice at 4 mg/kg/day (12 mg/m^2/day). Potential embryo-fetal toxicity has been evaluated in rats and rabbits. Alvocidib caused embryo-fetal toxicity at IV doses ≥0.32 mg/kg/day (1.9 mg/m^2/day) in Sprague-Dawley rats and ≥0.1 mg/kg/day (1.2 mg/m^2/day) in rabbits. Toxic effects included intrauterine deaths, decreased intrauterine fetal growth, and skeletal changes (ie, decreased ossification and malformations). In a peri- and postnatal toxicity study in rats, toxicities were limited to bilateral microphthalmia in one 0.1 mg/kg/day (0.6 mg/m^2/day) F1 pup and unilateral eye microphthalmia in one 0.75 mg/kg/day (4.5 mg/m^2/day) F1 pup. These malformations were considered to be test-article related based on historical control data in this strain of rats.

2.4.7 Other Toxicity Studies

In the in vitro hemolytic potential assay, alvocidib formulations at final concentrations of >1 mg/mL induced slight to severe hemolysis of human whole blood. Alvocidib formulations caused precipitates at final concentrations of ≥2.5 mg/mL and cloudiness at a final concentration of 1 mg/mL when mixed with human plasma in vitro. Final alvocidib concentrations of ≤0.5 mg/mL and the vehicle did not cause hemolysis in human whole blood and were fully compatible with human plasma in vitro.

Alvocidib did not produce in vitro phototoxicity in 3T3 cells when tested up to 200 µg/mL (limit of solubility in the culture media) in the presence of ultraviolet A irradiation.

2.5 CLINICAL STUDIES

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

2.5.1 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) [16]. Investigators at the University of Maryland, and then at Johns Hopkins, added alvocidib to AM for the dual purpose of initial cytoreduction and enhancing the cell cycle progression of the remaining leukemic cell cohort, followed by the

cycle-dependent agents cytarabine and mitoxantrone (FLAM regimen). Two alvocidib dosing schedules have been evaluated: by 1-hour *bolus* infusion, and by a *hybrid* dosing schedule consisting of a 30-minute short IV bolus dose followed by a 4-hour IV infusion. A listing of all eight (8) clinical studies of FLAM in relapsed/refractory patients and newly diagnosed patients is provided in Table 1.

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

Study (Reference)	N	Treatment Regimen	Patient Population
Study 1: JHOC J0254/ NCI-3170 Phase 1 FLAM [17]	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 [18]	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 [19]	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 [20]	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 [21]	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 [22] *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 [23]	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 [24] *Ongoing follow-up*	AML Total: 165 FLAM: 109 7&3: 56	Arm A: *Bolus FLAM* Alvocidib Bolus 1-hr IV: 50 mg/m^2/d on Days 1-3 Ara-c: 2 gm/m^2/72h by CIV given Days 6-8 Mitoxantrone: 40 mg/m^2 given by 60-120 min IV on day 9 Arm B: 7&3 Ara-c: 100 mg/m^2/day IV infusion Days 1-7 Daunorubicin 90 mg/m^2 /day IV over 30-60 minutes Days 1, 2, 3	Adults median age 60 (FLAM), newly diagnosed, pathologically confirmed, previously untreated intermediate/poor risk AML (including Secondary AML)

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

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

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

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

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

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

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

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

2.6 JUSTIFICATION FOR STUDY TREATMENT PLAN

About 30% of patients diagnosed with MDS will progress to AML. Patients with IPSS-R intermediate and above MDS have an increased risk of developing AML and may be treated with the same chemotherapy regimens used in patients with AML.

Two alvocidib dosing schedules (ie, bolus and hybrid dosing) have been evaluated in eight clinical trials in patients with AML (Table 1). While both regimens of ACM 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 [23]. A high CR rate (39%) has also been documented with the hybrid regimen in relapsed and refractory AML patients (and 92% CR among relapsed-only patients) [21]. 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.

Alvocidib is a potent inhibitor of CDK9 and downregulates CDK9-driven transcription of super enhancer-regulated genes, such as c-Myc and MCL-1. Primary pharmacology of alvocidib is related to potent inhibition of CDK9 which disrupt super enhancer-driven expression of MCL-1 as this plays a central role in the super enhancer complex. The ability of alvocidib to alter the expression of MCL-1 may provide a novel approach to targeting MCL-1-dependent malignancies such as MDS or multiple myeloma. Apoptosis following alvocidib is rapid and not linked to cell cycle arrest [25].

In the clinic, three-day dosing of alvocidib resulted in an average reduction of circulating blasts of greater than 75% [19]. A functional assay has been developed to identify patients with a malignancy dependent on MCL-1 which involves adding NOXA peptide or a NOXA mimetic peptide (eg, T-MS1) to patient samples and measuring induction of apoptosis. The readout is percentage of cells entering apoptosis. In MDS patients, the assay is being planned to be run on peripheral blood as well as standard bone marrow aspirates on diagnostic bone marrow samples. This functional assay is scalable and has a quick turnaround (24-48 hours).

Enrollment in this study to date has not been optimal and seems to be driven, in part, by the availability of slots for patient participation. Timing for initiation of treatment is critical in these patients and sometimes these patients are hesitant to wait 14 or more days to begin therapy. A modified enrollment strategy implemented in Amendment 2 permits the first two patients in a dose cohort to be enrolled simultaneously with the third patient permitted after Day 14 if no unacceptable toxicities are observed in the first two patients. This

change will not compromise patient safety and will permit more efficient escalation to planned Dose Cohort 4 which was the established MTD in prior AML studies with alvocidib.

The hypomethylating agents azacitidine and decitabine are used for the treatment of patients with MDS in the US. On a global basis, azacitidine is approved and used. It is now the intent to have a global experience with the combination of azacitidine and alvocidib in patients with MDS. In efforts to reduce the amount of time patients are required to be at the clinic, alvocidib administration in this amendment will change from hybrid dosing (ie, 30-minute IV bolus followed 30 minutes later by a 4-hour IV infusion [IVI]) to a ***30- to 60-minute*** IVI which has been tested before. Once the MTD of alvocidib administered using hybrid dosing is determined, 2 cohorts of patients (at least 3 patients each) will receive the standard dose of azacitidine (75 mg/m^2) followed by alvocidib at a starting dose of 75 mg/m^2/day administered by ***30- to 60-minute*** IVI with escalation to 90 mg/m^2/day in the absence of dose-limiting toxicities. Alvocidib has previously been administered as a ***30- to 60-minute*** IVI at a dosage of 78 mg/m^2/day to patients with solid tumors who had been previously treated (some heavily) [31]. Observed Grades 3 and 4 adverse events (AEs) included lymphocytopenia, neutropenia (dose-limiting), diarrhea, hypophosphatemia, and reversible hyperglycemia. Since patients in the current study will likely be treated prophylactically with antibiotics, antivirals, and antifungal therapies (depending on each institution's standards of care) and will receive aggressive antidiarrheal treatment, similar AEs should be manageable. Once the MTD of alvocidib administered as a ***30- to 60-minute*** IVI has been determined in those 2 cohorts, up to 25 patients will be enrolled in an Expansion cohort to receive either HMA followed by alvocidib to further characterize the safety of the combinations and evaluate potential signals of alvocidib activity.

2.7 SUMMARY OF RISK AND BENEFITS

While there have been no clinical studies conducted using alvocidib in sequence with decitabine (DEC) or azacitidine (AZA) in this patient population, the safety profile for alvocidib—either as a single agent or in combination with cytarabine and mitoxantrone—has been well described in several clinical studies in patients with AML and appears to be acceptable. Alvocidib has been administered in combination with vorinostat in patients with relapsed, refractory, or poor prognosis acute leukemia or refractory anemia with excess blasts-2 [26]. Side effects were tolerable and manageable with 50% of patients experiencing stable disease. Since the outcome in patients with high-risk MDS is poor, we hope to evaluate whether treatment with alvocidib in sequence after DEC ***(during dose escalation)*** or AZA enacts any enhanced antileukemic and/or pharmacokinetic effects in this elderly patient population.

3. STUDY OBJECTIVES

3.1 PHASE 1B OBJECTIVES

Primary:

- To determine the maximum tolerated dose (MTD) and recommended Phase 2 dose (RP2D) of alvocidib when administered in sequence after DEC ***(during dose escalation)*** or AZA in patients with previously untreated MDS and patients with MDS who have received <6 cycles of treatment with previous hypomethylating agents (HMAs)

Secondary:

- To determine the Complete Response Rate ([CRR]: complete response [CR] / complete response with incomplete blood count recovery [CRi] / CRmarrow / partial response [PR] / hematologic improvement [HI])

- To determine if treatment with alvocidib administered in sequence after DEC ***(during dose escalation)*** or AZA results in improvements in transfusion dependence (defined as an average transfusion requirement of ≥2 units/28 days of RBCs without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose) and/or hemoglobin level (transfusion data to include the hemoglobin value for which the transfusion was administered and must have been ≤10.0 g/dL within 7 days prior to transfusion administration to be counted toward the RBC transfusion-dependent status):
    - Hemoglobin increase of ≥1.5 g/dL without erythropoietin-stimulating agents (ESAs)

      OR
    - Reduction of ≥4 RBC transfusions/8 weeks in comparison to pre-treatment values and lasting at least 8 weeks

Exploratory:

- To evaluate MCL-1 dependence in untreated MDS patients via BH3 profiling

- To determine whether DEC ***(during dose escalation)*** or AZA treatment modulates MCL-1 dependence in peripheral blood

3.2 PHASE 2 OBJECTIVES

Primary:

- To determine preliminary efficacy and anti-MDS activity of alvocidib administered in sequence after AZA in untreated patients with de novo or secondary MDS

Secondary:

- To assess the CRR (per 2006 revised International Working Group [IWG] criteria [28])
- To evaluate the tolerability of alvocidib when administered in sequence following AZA in this patient population
- To determine whether a defined BH3 profile predicts response to alvocidib administered in sequence after AZA in untreated MDS population
- To determine if treatment with alvocidib administered in sequence after AZA results in improvements in transfusion dependence (defined as an average transfusion requirement of ≥2 units/28 days of RBCs without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose) and/or hemoglobin level (transfusion data to include the hemoglobin value for which the transfusion was administered and must have been ≤10.0 g/dL within 7 days prior to transfusion administration to be counted toward the RBC transfusion-dependent status):
    - Hemoglobin increase of ≥1.5 g/dL without ESAs

      OR
    - Reduction of ≥4 RBC transfusions/8 weeks in comparison to pre-treatment values and lasting at least 8 weeks

<u>Exploratory Objectives</u>

- To assess the pharmacokinetics (PK) of alvocidib when administered in sequence after AZA
- To evaluate correlative biomarkers of therapy including, but not limited to, BH3 profiling with an emphasis on MCL-1 dependence, genetic mutations, and other biomarkers associated with MDS
- To document the percentage of patients transplanted post-treatment and outcomes post-transplant

Additional exploratory analyses may be performed, if useful, in the interpretation of the data.

4. INVESTIGATIONAL PLAN

4.1 OVERALL STUDY DESIGN

This is a Phase 1b/2, open-label, safety, efficacy, PK, and pharmacodynamic (PD) study.

Phase 1b

Patients will be enrolled in cohorts of 3-6 patients. Escalation of the alvocidib dose will follow a standard 3+3 design with sequential cohorts of 3 patients treated with incrementally higher doses of alvocidib administered in sequence after DEC ***(during dose escalation)*** or AZA until a DLT is observed and the MTD is established (*Section 4.5.3*). The first 2 patients at a dose level may be enrolled simultaneously and the third patient will be enrolled after 14 days so long as the 2 initial patients have not experienced any unacceptable toxicity.

If 1 of 3 patients in a cohort experiences a DLT, up to 3 additional patients will be treated at that dose level. If no additional DLTs are observed in the expanded 3- to 6-patient cohort within 28 days after the last patient was first dosed, the 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. Adverse events meeting the definition of DLT during Cycles 2+ will be taken into consideration when evaluating dose escalation.

Once the MTD or preliminary RP2D of alvocidib administered via hybrid dosing (ie, IV bolus followed by IVI) is identified, 2 cohorts of at least 3 patients each will receive AZA followed by alvocidib administered as a ***30- to 60-minute*** IVI.

Expansion at MTD

Once the MTD or preliminary RP2D of alvocidib administered as a ***30- to 60-minute*** IVI is determined, up to 25 patients will be enrolled in an Expansion cohort to receive alvocidib following AZA to confirm safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity. Once this Expansion cohort is completed, the study will progress to Phase 2.

Phase 2

The Phase 2 design is based on the Simon 2-stage minimax design [27].

- Stage 1: Up to 15 evaluable patients will be enrolled and treated at the RP2D identified in the Phase 1b study. Stage 2 may be initiated at any point after confirming a response (CR/CRi/CRmarrow/PR/HI) in two Stage 1 patients. If there is ≤1 responder among 15 evaluable Stage-1 patients, the study will be stopped after Stage 1.

- Stage 2: Ten patients will be enrolled to bring the total enrollment in Phase 2 (including Stage-1 patients) to 25 evaluable patients. Stage-2 patients will also receive the RP2D dose of alvocidib administered by ***30- to 60-minute*** IVI identified in the Phase 1b study. If 6 or more responses are observed in 25 patients, the conclusion will be that the combination regimen is worthy of further investigation. When the true response rate of 30% (alternative hypothesis) is tested against the null hypothesis response rate of 10%; this design yields a Type I error rate of 0.05 and power of 80%.

Any patient who withdraws from Stage 1 or 2 for treatment-related toxicity or disease progression, or dies prior to being evaluated for response will be considered a nonresponder. Patients who drop out for other reasons prior to being assessed for response will be considered unevaluable and may be replaced. Enrollment into Phase 2 may be stopped at any point once ≥6 patients have had a response to treatment, but the maximum enrollment in Phase 2 will be 25 evaluable patients.

The study may be stopped early during Phase 2 in the event of unacceptable toxicities. Early stopping rules for safety are based on a Sequential Probability Ratio Test (SPRT) with a baseline toxicity rate of 5%; an upper ceiling of unacceptable toxicity of 20% (ie, toxicities meeting one or more DLT criteria as stated in *Section 4.5.3*); and alpha=0.05 with power=80%.

The study will be stopped if unacceptable toxicities are observed in:

- 2 of the first 2 patients
- 3 of the first 11 patients
- 4 of the first 20 patients
- 5 of the first 29 patients (Ph 2 study to enroll maximum of 25 patients, but considering possible replacement patients)

Safety Assessments

Incidence rates of treatment-emergent adverse events (TEAEs) will be summarized within treatment group at the Medical Dictionary for Regulatory Activities (MedDRA) preferred term and primary system organ class levels.

Similar summaries will be made for subsets of adverse events (AEs) such as (1) those judged by the Investigator to be related to study treatment, and (2) serious adverse events (SAEs).

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

Response Assessments

Response to treatment will be assessed according to the revised IWG Criteria [28].

4.2 ASSIGNMENT TO TREATMENT

This is an open-label study. All patients will receive alvocidib and DEC ***(during dose escalation)*** or AZA according to the dose cohort in which they are enrolled. The MTD will be assessed during the first cycle of treatment though toxicities observed during Cycle 2 and later cycles will be considered when determining the RP2D and the dose escalation scheme.

Patients will be enrolled into open dosing cohorts from all participating centers. The study will be managed by the Sponsor and/or its designee and all sites must receive authorization from the Medical Monitor for enrollment of any eligible patient.

4.3 PATIENT POPULATION

The targeted patient population will generally fall into one of these categories:

- Patients with previously untreated MDS
- Patients with MDS who have received <6 cycles of treatment with hypomethylating agents (HMAs)
- Patients with de novo (cause unknown) or secondary MDS (treatment-related) who are not eligible for intensive induction chemotherapy or stem cell transplant
    - All French-American-British (FAB) subtypes (refractory anemia, refractory anemia with ringed sideroblasts, refractory anemia with excess blasts, refractory anemia with excess blasts in transformation, and chronic myelomonocytic leukemia)
    - Intermediate and above per the revised International Prognostic Scoring System (IPSS-R) groups

4.3.1 Number of Patients

It is planned to enroll up to 24 patients in the Phase 1b, dose-escalation part of this study to ***determine*** the MTD/RP2D. Once the MTD or preliminary RP2D of alvocidib administered via hybrid dosing (ie, IV bolus followed by IVI) is identified, 2 cohorts of patients (minimum of 6 patients; 3 per cohort) will receive AZA followed by alvocidib administered as a ***30- to 60-minute*** IVI.

Once the MTD or preliminary RP2D of alvocidib administered as a ***30- to 60-minute*** IVI is determined, up to 25 patients will be enrolled into an Expansion cohort to receive alvocidib following AZA to further characterize the safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity. Once this Expansion cohort is completed, the study will progress to Phase 2 with planned enrollment of up to 25 patients (Stage 1 and Stage 2) to confirm the efficacy of alvocidib when administered with AZA. Total study time (Phase 1b and Phase 2) will be approximately 36 to 44 months.

4.3.2 Inclusion Criteria

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

1. Aged ≥18 years

2. Phase 1b ***Dose Escalation***: Patients with previously untreated MDS and patients with MDS who received fewer than six (6) cycles of previous HMAs

   Phase 1b Expansion: Untreated patients with de novo or secondary MDS

   Phase 2: Untreated patients with de novo or secondary MDS

3. Patients with an Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) score ≤2 at enrollment

4. 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.)

5. Patients with a life expectancy of ≥3 months (90 days)

6. Patients with adequate major organ functions meeting the following criteria on the basis of laboratory data within 4 weeks (28 days) before enrollment (if multiple data are available, most recent data during the period):

   a. Serum creatinine: ≤1.8× the upper limit of the normal (ULN) range

   b. Total bilirubin: ≤2× the ULN

   c. Aspartate transaminase (AST) and alanine transaminase (ALT): ≤3× the ULN

7. Be able to comply with the requirements of the entire study.

8. Patients with Revised International Prognostic Scoring System (IPSS R) intermediate-, high-, and very high-risk MDS

4.3.3 Exclusion Criteria

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

1. Presence of concomitant severe cardiovascular disease:

    a. Patients who had myocardial infarction within 6 months (180 days) before enrollment

    b. Patients with significant diseases at enrollment that may affect study treatment, such as New York Heart Association (NYHA) Functional Class III or IV heart disease, National Cancer Institute (NCI) Common Toxicity Criteria for Adverse Events (CTCAE) v5.0 grade ≥3 arrhythmia, angina pectoris, abnormal electrocardiogram findings, interstitial pneumonia or pulmonary fibrosis

2. Presence of concomitant malignancy requiring chemotherapy or any malignancy (except basal and squamous cell carcinoma of the skin) for which the patient received chemotherapy within 6 months prior to enrollment. NOTE: Diagnosis of any previous or concomitant malignancy is, thus, not an exclusion criterion.

3. Presence of uncontrolled or uncontrollable infection(s); or ≥Grade 3 infection according to NCI CTCAE v5.0

4. Presence of any psychological, familial, sociological or geographical condition that, in the opinion of the investigator, could potentially hinder compliance with the study protocol and follow-up schedule

5. Patients with a dry tap on bone marrow aspiration before enrollment

6. Patients with concurrent autoimmune disease or a history of chronic or recurrent autoimmune disease, or patients who require long-term systemic steroid therapy greater than the equivalent of 20 mg of prednisone daily (excluding therapy given on an ‘as needed’ (PRN) basis)

7. Patients with other documented malignancies within the past year aside from synchronous or metachronous multiple cancers with a disease-free period of ≤5 years (excluding carcinoma in situ, mucosal carcinoma, or other such carcinomas curatively treated with local therapy)

8. Patients with ≥Grade 2 hemorrhage according to NCI CTCAE v5.0

9. Patients who have previously received alvocidib or another cyclin-dependent kinase 9 (CDK9) inhibitor
10. Patients who are pregnant or breastfeeding
11. Female patients of childbearing potential who are sexually active and unwilling to use a medically acceptable method of contraception associated with a low failure rate during and for at least 6 months after the last dose of study drug. (Patients will be considered to be of childbearing potential unless surgically sterilized by hysterectomy, or bilateral tubal ligation/ salpingectomy, or postmenopausal for at least 2 years.)
12. Male patients with partners of childbearing potential who are unwilling to use condoms in combination with a second effective method of contraception during the trial and for at least 6 months after the last administration of study treatment.
13. Patients who are inappropriate for participation in the study for other reasons in the opinion of the investigator or sub-investigator(s)
14. Patients with a known hypersensitivity to ***DEC (those patients enrolled in dose escalation)***, AZA***,*** or mannitol
15. ***Patients who have received erythropoietin-stimulating agents (ESAs) within 2 weeks (14 days) prior to Cycle 1/Day 1***

4.4 STUDY TREATMENT

4.4.1 Calculation of Doses

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. Doses can be adjusted for changes in body weight of ±10% used in the calculation of the current dose.

4.4.2 Study Drug Administration

Evaluation of the safety and efficacy of alvocidib when administered in sequence after DEC ***(during dose escalation)*** or AZA will occur in three phases:

- Phase 1b ***Escalation***: 3+3 dose escalation design
- ***Phase 1b*** Expansion at MTD: once the MTD of alvocidib administered by ***30- to 60-minute*** IVI is determined in Phase 1b, up to 25 patients will be enrolled in an Expansion cohort to receive alvocidib following AZA to confirm safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity
- Phase 2: using the RP2D from Phase 1b, initiate Simon 2-stage minimax design

Decitabine and Alvocidib

- Days 1-5: Decitabine to be administered at as a 1-hour IVI (ie, starting dose for Cohort 1; see *Table 5* for assigned decitabine doses per treatment cohort).
- Day 6 and 7: Drug-free days
- Day 8: Alvocidib to be administered as a 30-minute (±10 minutes) IV bolus followed up to 30 minutes later by a 4-hour (±15 minutes) IVI (see *Table 5* for assigned alvocidib doses per treatment cohort).

Azacitidine and Alvocidib

Once the MTD of alvocidib administered via hybrid dosing (ie, 30-minute bolus followed by a 4-hour IVI) has been determined, 2 cohorts of patients (minimum of 6 patients; 3 per cohort) will receive AZA followed by alvocidib administered as a ***30- to 60-minute*** IVI. Azacitidine may be administered as either an IVI over 10 to 40 minutes or as a subcutaneous (SC) injection on either a 7-day or 5-2-2 schedule (see below). Regardless of which AZA schedule or route of administration is used, alvocidib will be given on Day 10 as a ***30- to 60-minute*** IVI. Choice of schedule and route of administration of AZA will be at the discretion of the investigator.

- AZA 7-day Schedule
  - Days 1-7: AZA to be administered as a 75 mg/m^2 IVI over 10 to 40 minutes or SC injection daily for 7 consecutive days
  - Days 8-9: Drug-free days
  - Day 10: Alvocidib to be administered as a ***30- to 60-minute*** IVI
- AZA 5-2-2 Schedule
  - Days 1-5: AZA to be administered as a 75 mg/m^2 IVI over 10 to 40 minutes or SC injection daily for 5 consecutive days
  - Days 6-7: Drug-free days
  - Days 8-9: AZA to be administered as a 75 mg/m^2 IVI over 10 to 40 minutes or SC injection daily for 2 consecutive days
  - Day 10: Alvocidib to be administered as a ***30- to 60-minute*** IVI

4.4.2.1 Phase 1b: Dose-escalation

Patients will receive alvocidib administered in sequence after fixed doses of either DEC or AZA using a standard 3+3 design according to the dose cohorts in *Table 5* and *Table 6*.

Table 5: Proposed Dose Escalation Schema (DEC + Alvocidib)

Dose Level[a]	Days 1-5, Decitabine	Day 8, Alvocidib [b]	
	1-hr IV infusion	30-min bolus	4-hr IV infusion
1	**20 mg/m^2**	**20 mg/m^2**	**20 mg/m^2**
2	20 mg/m^2	30 mg/m^2	30 mg/m^2
3	20 mg/m^2	30 mg/m^2	45 mg/m^2
4	20 mg/m^2	30 mg/m^2	60 mg/m^2

a It is possible for additional and/or intermediate dose levels to be added during the course of the study.
b Alvocidib to be administered first as a 30-minute (±10 minutes) IV bolus followed up to 30 minutes later by a 4-hour (±15 minutes) IVI.

Two separate cohorts of patients (minimum of 6 patients; 3 per cohort) will receive AZA followed by alvocidib administered as a ***30- to 60-minute*** IVI according to the dose cohorts in Table 6.

Table 6: Proposed Dose Schema (AZA + Alvocidib)

Dose Level [a]	Azacitidine [b]	Day 10, Alvocidib
	IVI or SC injection [c]	*30- to 60-min* IVI
1A	75 mg/m^2	75 mg/m^2
1B	75 mg/m^2	90 mg/m^2

a It is possible for additional and/or intermediate dose levels to be added during the course of the study.
b AZA can be administered on either a 7-day or 5-2-2 schedule.
c AZA may be given as an IVI over 10 to 40 minutes or an SC injection.

The first 2 patients at a dose level may be treated simultaneously and the third patient will be treated after 14 days so long as the initial 2 patients have not experienced any unacceptable drug-related toxicity. Once the last patient enrolled has completed Day 28 without observation of a DLT and the next higher alvocidib dose level has not yet been studied, the alvocidib dose will be increased in a new 3-patient cohort.

If 1 of 3 patients in a cohort experiences a DLT, up to 3 additional patients will be treated at that dose level. If no additional DLTs are observed in the expanded 3- to 6 patient cohort within 28 days after the last patient was first dosed, the 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. Adverse events meeting the definition of DLT during Cycles 2+ will be taken into consideration when evaluating dose escalation.

4.4.2.2 Expansion at MTD/RP2D

Once the MTD or preliminary RP2D of alvocidib administered as a ***30- to 60-minute*** IVI is identified, up to 25 patients will be enrolled in an Expansion cohort to receive alvocidib following AZA to confirm safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity.

Once this Expansion cohort is completed, the study will progress to Phase 2.

4.4.2.3 Phase 2

The Phase 2 study will then commence in a Simon 2-stage minimax design [27] using the RP2D of alvocidib administered as a ***30- to 60-minute*** IVI determined from the Phase 1b study to explore the efficacy of alvocidib when administered in sequence after AZA.

- Stage 1: Up to 15 evaluable patients will be enrolled and treated at the RP2D identified in the Phase 1b study. Stage 2 may be initiated at any point after confirming a response (CR/CRi/CRmarrow/PR/HI) in two Stage 1 patients. If there is ≤1 responder among 15 evaluable Stage-1 patients, the study will be stopped after Stage 1.

- Stage 2: Ten patients will be enrolled to bring the total enrollment in Phase 2 (including Stage-1 patients) to 25 evaluable patients. Stage-2 patients will also receive the RP2D dose of alvocidib administered as a ***30- to 60-minute*** IVI identified in the Phase 1b study.

Enrollment into Phase 2 may be stopped at any point once ≥6 patients have had a response to treatment, but the maximum enrollment in Phase 2 will be 25 evaluable patients.

The study may be stopped early during Phase 2 in the event of unacceptable toxicities. Early stopping rules for safety are based on a Sequential Probability Ratio Test (SPRT) with a baseline toxicity rate of 5%; an upper ceiling of unacceptable toxicity of 20% (ie, toxicities meeting one or more DLT criteria as stated in *Section 4.5.3*); and alpha=0.05 with power=80%.

The study will be stopped if unacceptable toxicities are observed in:

- 2 of the first 2 patients
- 3 of the first 11 patients
- 4 of the first 20 patients
- 5 of the first 29 patients (Ph 2 study to enroll maximum of 25 patients, but considering possible replacement patients)

4.5 MANAGEMENT OF TOXICITIES AND DOSE 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.

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

Adverse events that are moderate to severe in intensity (see ***Section 8.1 for intensity assessment when NCI CTCAE grade [***Appendix D***] is not available***) and considered possibly or probably related to study drug treatments may result in the delay or termination of study treatment in affected patients. 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.

4.5.1 Management of Nonhematologic Toxicities

4.5.1.1 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. Preventative measures to reduce the likelihood of developing TLS include ensuring adequate hydration of patients prior to administration of alvocidib as well as careful monitoring of laboratory parameters before and after infusion. Investigators should follow their own institutional protocols in determining the best treatment for patients with symptoms of TLS.

4.5.1.1.1 TLS Prophylaxis for DEC + Alvocidib

Tumor lysis syndrome prophylaxis for patients receiving alvocidib in sequence following DEC should follow each institution's standard of care.

4.5.1.1.2 TLS Prophylaxis for AZA + Alvocidib

All patients receiving alvocidib in sequence following AZA will receive TLS prophylaxis as detailed below:

- IV Hydration
  - Prior to AZA
    - IV hydration according to each institution's standard of care

- Prior to Alvocidib
  - Mandatory IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 500 cc for 1-2 hours prior to alvocidib, then an additional 500 cc for 1-2 hours after alvocidib during Cycle 1 (volume may be reduced to between 250 cc-500 cc, if clinically indicated). Hydration is optional for subsequent cycles.

- Diarrhea Prophylaxis
  - During AZA and Alvocidib Dosing
    - Replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated.
  - Alvocidib-specific Measures
    - Alvocidib can induce diarrhea when given over a short period of time. Over-the-counter measures are typically effective in this setting if initiated early (see *Section 4.5.1.2*).
    - Persistent diarrhea despite optimal outpatient management would trigger medical consultation. Early consideration should be given for possible *Clostridioides difficile* (*C. difficile*) infection in this patient population and identifying/treating as expeditiously as possible is imperative (see *Section 4.5.1.2*).

- Oral Allopurinol
  - Mandatory oral allopurinol to be started on Day 1 of Cycle 1 and continued daily for first 2 weeks (ie, 14 days). This may be discontinued for subsequent treatment cycles if uric acid levels are within normal limits and there is no evidence of TLS.

- Oral Phosphate Binder
  - Mandatory oral phosphate binder to be started on Day 1 of Cycle 1 and continued daily for the first 2 weeks (ie, 14 days).
  - If serum phosphorus levels are <3 after the first treatment with alvocidib and there is no evidence of TLS, phosphate binders may be discontinued. Patients should continue to be monitored for TLS as outlined for subsequent treatment cycles. Caution is warranted for patients who still have a high blast count as they remain at risk for TLS with subsequent treatments.

- Laboratory Evaluations for Tumor Lysis Syndrome ('tumor lysis labs')
    - Tumor lysis labs include electrolytes (sodium, potassium, chloride, and carbon dioxide) as well as creatinine, calcium, lactate dehydrogenase (LDH), uric acid, and phosphorus levels
    - During Cycle 1
        - Monitor tumor lysis labs prior to AZA and 2 hours (±30 minutes) after completion of AZA dosing
        - Monitor tumor lysis labs prior to alvocidib IVI and 2 hours (±30 minutes) after completion of IV hydration post alvocidib.
        - All tumor lysis labs should be drawn, however the potassium level obtained at 2 hours post dose (AZA) and 2 hours post hydration (alvocidib) should be reviewed immediately to determine if additional treatment is warranted.
        - Labs will also be drawn daily for the first 2 days following alvocidib (ie, Days 11-12) and at least weekly for the remainder of Cycle 1
    - During Cycle 2, tumor lysis labs will be assessed:
        - Prior to C2D1 first AZA dose and at 2 hours post C2D1 AZA dose
        - Prior to C2D10 alvocidib IVI and 2 hours (±30 minutes) after completion of alvocidib IVI.
    - During Cycles 3+, tumor lysis labs will be checked at the discretion of the investigator in relation to patient blast counts

4.5.1.1.3 Risk of TLS with Alvocidib and Guidelines for Management

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 with the hybrid dosing regimen of alvocidib. 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 the hybrid dosing regimen of 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 (1 ampule) of IV dextrose 50%, unless there are other likely causes of hyperkalemia other than TLS or contraindication to its use. Investigators are strongly encouraged to consider patient hospitalization for inpatient monitoring and follow up.

- 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.

4.5.1.2 Diarrhea

Alvocidib can induce diarrhea when given over a short period of time. Dehydration from diarrhea can exacerbate the morbidity associated with tumor lysis syndrome (ie, acute renal failure). Over-the-counter measures are typically effective in this setting if initiated early. It is strongly suggested that patients take 2 tablets of loperamide, 2 mg each (or equivalent), prior to the alvocidib IVI and then take 1 tablet (2 mg) for every loose stool up to a maximum of 8 tablets (16 mg) in a 24-hour period. 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, diphenoxylate hydrochloride with atropine sulfate (or equivalent) 5 mg orally four (4) times daily may be added. If diarrhea is not controlled with the above prophylactic regimens and is grade 2 or greater, patients should contact the clinic and study drug treatment should be held until diarrhea has resolved. Should diarrhea persist beyond Cycle 1, patients should undergo testing for *C. difficile*. Should testing indicate the presence of *C. difficile*, appropriate antibiotics targeting this infection should be initiated. Should testing exclude the presence of *C. difficile*, diarrhea prophylaxis similar to Cycle 1 should be continued in subsequent cycles. Replacement of excessive fluid losses should be done unless otherwise clinically indicated.

4.5.1.3 Nausea/Vomiting

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

4.5.1.4 Infection Prevention

Prophylactic antibiotic, antiviral, and/or antifungal therapy is left to the discretion of the treating physician and according to institutional standards.

Routine growth factor support is not allowed before achieving a leukemia-free bone marrow state. Support with growth factors (eg, filgrastim) is discouraged. If a patient has Grade 4 sepsis with life-threatening infection with ongoing neutropenia, then this may be considered at the discretion of the Investigator.

4.5.2 Management of Hematologic Toxicities

Bone marrow suppression and associated complications are expected events during MDS therapy and are part of the treatment process (marrow emptying of abnormal 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.

4.5.3 Dose-limiting Toxicities

Dose-limiting toxicities (DLTs) will be determined during Cycle 1 and defined as follows based on the NCI CTCAE v5.0:

- Any Grade 5 toxicity that is not clearly and incontrovertibly related to the underlying disease or extraneous causes
- Any Grade 4 nonhematologic toxicity considered at least possibly drug related
- Grade 4 neutropenia lasting ≥42 days from the start of a cycle in the absence of evidence of active disease
- Any AST or ALT elevation ≥3x ULN accompanied by serum bilirubin levels >2x ULN
- 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 bilirubin, AST, ALT or alkaline phosphatase will be considered dose-limiting only if resolution to ≤Grade 2 requires more than 7 days

- 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 3 creatinine elevation that does not resolve to ≤Grade 2 within 7 days
- Anorexia, fever, neutropenic fever, and infections of any grade

- Bone marrow hypoplasia that occurs for >42 days with bone marrow (BM) cellularity ≤5% and no evidence of MDS/leukemia. Bone marrow disease assessments conducted per protocol on Day 28 (±3 days) of every even cycle may be used to guide this decision.

4.5.4 Dose Modifications

Doses of DEC, AZA, and alvocidib will not be reduced during Cycle 1. In patients who receive multiple treatment cycles, the alvocidib dose may be reduced by one dose level based on the observed toxicity that occurred during the preceding cycle. If further toxicities occur during one or more cycles at the new reduced dose level, no further reductions will be permitted, and the patient should be discontinued from the study.

No dose re-escalations will be allowed for any patient who had a previous dose reduction due to toxicity or delayed recovery.

In the event of toxicity in Cycles 2+, the dose of DEC or AZA may be adjusted according to the guidelines set forth in the US Prescribing Information (USPI) for each drug [29, 30]. Guidelines for dose adjustments of alvocidib are provided in *Table 7*.

Table 7: Guide to Alvocidib Dose Adjustments Based on Toxicities

Drug-related AE Grade	Action Regarding Alvocidib Dosage
Grade 1	Current dose level
Grade 2	Investigator's option to reduce dose by 1 dose level with agreement of the Medical Monitor
Grade 3*	Withhold, then reduce dose by 1 dose level upon recovery to ≤Grade 1 or baseline with agreement of the Medical Monitor.
Grade 4	Investigator and Medical Monitor review to determine if patient may continue on study with appropriate dose reduction upon recovery to ≤Grade 1 or baseline.

* Excluding brief (based on the investigator's judgment) Grade 3 vomiting or diarrhea with suboptimal management. In addition, Grade 3 toxicities must also meet the definition of a DLT.

Patients who experience a DLT will be required to discontinue study participation, unless the Investigators and Medical Monitor determine that it is in the best interest of the patient to continue with the dose reduction and only upon recovery of the toxicity to Grade 2 or better.

Dose reduction will be required for patients who have a delay in treatment greater than 6 weeks due to a lack of recovery of any hematologic or nonhematologic toxicity, even if DLT criteria are not met. Subsequent retreatment of patients who are not able to be treated after a 6-week delay and who eventually recover will be discussed between Investigators and Medical Monitor considering the potential benefit/risk for the individual patient. In addition, dose reductions may be permitted for patients who have toxicities that do not meet the criteria of a DLT. These toxicities will be discussed by the Investigators and Medical Monitor to determine if it would be in the best interest of the patient to continue to receive decitabine ***or azacitidine*** and a reduced dose of alvocidib.

4.6 CONCOMITANT MEDICATIONS AND THERAPIES

4.6.1 Previous Therapies

During Screening, patients will be asked about all previous therapies and all medications used during the previous 28 days from anticipated first dose. This information will be recorded in the source documentation and appropriate CRF along with the diagnosis or reason for use. If a branded product is being taken, the generic name should be reported, if known.

Subjects will not be enrolled into the study if they have had any previous treatment with alvocidib or other CDK inhibitor.

4.6.2 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 28 days 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.

4.6.2.1 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 PI. 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 Phase, but not within 12 hours prior to dosing.

4.6.2.2 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.
- Live vaccines within 14 days prior to first study drug administration, during the study, and for approximately 3 months after the last dose of study drug.
- ***Routine growth factor support is not allowed. Growth factor support can be given at the discretion of the Investigator and with the Medical Monitor's approval in the presence of life-threatening infection with ongoing neutropenia.***

4.6.3 Birth Control Requirements for Fertile Patients

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

4.7 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 PI (or a responsible, appropriately trained and credentialed professional[s] designated by the PI). In the event of a significant deviation from the protocol due to an emergency, accident or error, the PI or Designee must contact the Sponsor at the earliest possible time by telephone. This will allow an early joint decision to be made as to whether or not the patient should continue in the study. This decision will be documented in writing by both the PI and the Sponsor.

5. ON-STUDY CLINICAL AND LABORATORY EVALUATIONS

See *Appendix A-1* – Schedule of Activities (Patients Receiving DEC and Alvocidib), Phase 1b, Expansion, and Phase 2; *Appendix A-2* – Schedule of Activities (Patients Receiving AZA and Alvocidib), Phase 1b, Expansion, and Phase 2; and *Appendix A-3* – Schedule of TLS Evaluations (Patients Receiving AZA and Alvocidib).

During each cycle, it is critical to record all laboratory values for hematology and serum chemistry parameters collected during the time the patient is on study to facilitate change-from-baseline analyses. Any laboratory parameters assessed outside the per-protocol visits (ie, interim labs) will be recorded as 'Unscheduled Laboratory Assessments' in the database.

5.1 PREDOSE ASSESSMENTS (PHASE 1B, EXPANSION AT MTD, AND PHASE 2)

5.1.1 Within 28 Days Prior to First Dose of Cycle 1

Perform the following activities and evaluations within 28 days prior to administration of the first dose of study drug:

- Obtain written informed consent (must be obtained prior to study-related screening evaluations. Evaluations performed as standard-of-care prior to obtaining consent may be utilized for screening)
- Collect and document a complete medical and disease history including histologically confirmed diagnosis of MDS
- Collect and document transfusion dependency, ie, transfusion requirement without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose and/or hemoglobin level
    - ***Record all transfusions (hemoglobin and platelet) for the 8 weeks/56 days prior to screening (including the transfusion date and number of units with each transfusion)***
    - ***Record all CBC data for the 8 weeks/56 days prior to screening including each CBC that prompted a transfusion***
- Perform a complete physical examination of all body systems and including height (cm) and weight (kg)
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Assess Eastern Cooperative Oncology Group (ECOG) Performance Status (PS) (*Appendix B*)
- Perform a 12-lead electrocardiogram (ECG) including assessment of corrected QT interval
- Perform chest radiograph ('x-ray') (may omit, if performed within 28 days prior to anticipated first dose)

- Collect blood for evaluation of laboratory parameters (*Appendix E*):
  - Hematology
  - Full serum chemistry panel
  - Coagulation panel
- Collect urine or serum sample for beta-human chorionic gonadotropin (β-hCG) pregnancy test for females of child-bearing potential
- Perform bone marrow biopsy and/or aspiration and collect peripheral blood for disease status, standard cytogenetics, and pharmacodynamic (PD) analyses (*Section 7.4*, *Table 9* and *Table 10;* ***Appendix E***).
  - If the initial bone marrow aspirate is nonproductive or not diagnostic, the procedure must be repeated.
  - Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.
- Record all concomitant medications including all prescription drugs, nonprescription drugs, and nutritional supplements within the past 28 days

5.1.2 Within 72 Hours Prior to First Dose of Cycle 1

Perform the following activities and evaluations anytime within 72 hours prior to administration of the first dose (on Day 1) of study drug:

- Perform a complete physical examination including weight (kg), calculation of BSA, and baseline signs and symptoms
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Assess ECOG PS (*Appendix B*)
- Collect blood for evaluation of laboratory parameters (*Appendix E*):
  - Hematology panel
  - Full serum chemistry panel
- TLS prophylaxis for patients receiving DEC ***(during dose escalation)*** followed by alvocidib should follow each institution's standard of care.
- 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 taken since previous predose assessment

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

As early as possible during the screening process, the Medical Monitor (or designee) should be provided the required information for review and approval to enroll patient with updates provided, if applicable, up to the point of enrolling the patient. See Study Manual for detailed instructions on procedures on enrollment.

5.2 TREATMENT ASSESSMENTS DURING PHASE 1B AND PHASE 2

5.2.1 Cycle 1

5.2.1.1 Cycle 1 – Day 1 (and as otherwise indicated)

- Patients receiving DEC ***(during dose escalation)*** and Alvocidib
    - Initiate TLS prophylaxis and supportive care measures according to each institution's standard of care
- Patients receiving AZA and Alvocidib
    - Administer pretreatment IV hydration prior to first AZA dose per institutional standards
    - Begin oral allopurinol daily from Days 1-14 of Cycle 1
    - Begin oral phosphate binder daily from Days 1-14 of Cycle 1 (see *Section 4.5.1.1.2*)
    - Replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated (see *Section 4.5.1.2* and *Section 4.5.1.3*)
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures) prior to first dose
- Obtain baseline signs and symptoms prior to first dose
- Collect blood for evaluation of laboratory parameters (*Appendix E*):
    - Hematology panel
    - Full serum chemistry panel
    - Tumor lysis labs required just prior to AZA dose in patients receiving AZA + alvocidib and 2 hours post AZA dose (*Appendix A-3*)
        - Immediately assess potassium level at 2 hours post dosing for indications of tumor lysis syndrome
        - Tumor lysis labs to also be drawn on Days 2 and 3 prior to AZA doses during Cycle 1
- Collect peripheral blood for PD analyses on Days 1, 3 and 5 in patients receiving DEC or on Days 1, 3, 5, and 7 in patients receiving AZA (*Section 7.4*, *Table 9* and *Table 10*, respectively)

- 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
- Administer prophylactic antibiotics, antivirals, antifungals, antiemetics, antidiarrheals according to standard practices at each investigational site
- Administer DEC on Days 1-5 ***(during dose escalation)*** or AZA on 7-day or 5-2-2 schedule according to *Section 4.4.2.1*
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.1.2 Cycle 1 – Day 8 (Patients receiving DEC + Alvocidib)
Cycle 1 – Day 10 (Patients receiving AZA + Alvocidib)

- Perform an abbreviated physical examination (AE- or symptom-directed exam)
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures) 5-15 minutes prior to initiation of alvocidib following a 5-minute rest
- Assess ECOG PS (*Appendix B*)
- Alvocidib-specific TLS Prophylaxis
    - Administer pretreatment IV hydration at least 2 hours prior to first dose of alvocidib
    - Continue oral allopurinol daily from Days 1-14 of Cycle 1
    - Continue oral phosphate binder daily from Days 1-14 of Cycle 1 (see *Section 4.5.1.1.2*)
    - Replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated (*Section 4.5.1.2* and *Section 4.5.1.3*)
- Administer prophylactic antibiotics, antivirals, antifungals, antiemetics, antidiarrheals according to standard practices at each investigational site
- Administer alvocidib according to *Section 4.4.2.1*

- Collect blood for evaluation of laboratory parameters just prior to the start of the infusion and again approximately 4 hours post end of IVI (*Appendix E*):
    - Hematology panel
    - Full serum chemistry panel
    - Tumor lysis labs required just prior to alvocidib dosing and 2 hours after end of IV hydration post alvocidib IVI in patients receiving AZA + alvocidib (*Appendix A-3*)
        - All tumor lysis labs should be drawn; however, the potassium level obtained at 2 hours post IV hydration should be reviewed immediately to determine if additional treatment is warranted (*Section 4.5.1.1.3*).
- Phase 1b only – Collect blood for evaluation of PK parameters (*Section 7.3, Table 8*)
- Collect peripheral blood for PD analyses (*Section 7.4, Table 9* and *Table 10*)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.1.3 Cycle 1 – Day 9 (Patients receiving DEC + Alvocidib)
Cycle 1 – Day 11 (Patients receiving AZA + Alvocidib)

- Perform an abbreviated physical examination (AE- or symptom-directed exam)
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Collect blood for follow-up evaluation of laboratory parameters (*Appendix E*):
    - Hematology panel
    - Full serum chemistry panel
        - Full panel required with specific attention to tumor lysis labs in patients receiving AZA + alvocidib on Day 11 (*Appendix A-3*)
- Patients receiving AZA and alvocidib
    - Continue oral allopurinol daily on Days 1-14 of Cycle 1
    - Continue oral phosphate binder daily from Days 1-14 of Cycle 1 (see *Section 4.5.1.1.2*)

- Phase 1b only – Collect blood for evaluation of PK parameters (*Section 7.3, Table 8*)
- Collect peripheral blood for PD analyses (*Section 7.4, Table 9* and *Table 10*)
- Assess patients for adequate hydration at all study visits and replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated (*Section 4.5.1.2* and *Section 4.5.1.3*)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.1.4 Cycle 1 – Day 12 (Patients receiving AZA + Alvocidib)

- Collect blood for follow-up evaluation of laboratory parameters (*Appendix E*):
  - Tumor lysis labs
- Continue oral allopurinol daily on Days 1-14 of Cycle 1
- Continue oral phosphate binder daily on Days 1-14 of Cycle 1
- Assess patients for adequate hydration at all study visits and replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated (*Section 4.5.1.2* and *Section 4.5.1.3*)

5.2.1.5 Cycle 1 – Days 15, 22 (±3 Days)

Perform the following activities and evaluations on Days 15 and 22 of Cycle 1:

- Perform an abbreviated physical examination (AE- or symptom-directed exam)
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Assess ECOG PS (*Appendix B*)
- Collect blood for follow-up evaluation of laboratory parameters (*Appendix E*):
  - Hematology panel
  - Full serum chemistry panel
  - Tumor lysis labs (Day 22 in patients receiving AZA + alvocidib)
- Collect peripheral blood for PD analyses (*Section 7.4, Table 9* and *Table 10*)

- Assess patients for adequate hydration at all study visits and replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated (*Section 4.5.1.2* and *Section 4.5.1.3*)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.2 Cycles 2+

5.2.2.1 Cycles 2+: At Least 2 Hours Prior to First Dose of Alvocidib in Patients Receiving AZA + Alvocidib

During Cycles 2+, pretreatment IV hydration, oral allopurinol, and oral phosphate binder may be discontinued in patients receiving AZA and alvocidib if serum uric acid and phosphorus levels are within normal limits and there is no evidence of TLS.

Should TLS be suspected during subsequent cycles, perform the following procedures at least 2 hours prior to alvocidib dosing on Day 10 (unless otherwise stated):

- Initiate supportive care measures prior to first dose of alvocidib in patients receiving AZA + alvocidib to minimize the likelihood of TLS:
  - Administer pretreatment IV hydration and determine if oral allopurinol and oral phosphate binder are still clinically indicated (see *Section 4.5.1.1.2* and *Appendix A-3*)
  - Assess patient for adequate hydration at all study visits and replace excessive fluid losses, including from diarrhea, unless otherwise clinically indicated (*Section 4.5.1.2* and *Section 4.5.1.3*)

5.2.2.2 Cycles 2+ – Day 1

Perform the following activities and evaluations on Day 1 of Cycles 2+:

- Perform an abbreviated physical examination including weight (kg) and calculation of BSA
- Assess ECOG PS (*Appendix B*)
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures) prior to first dose
- Collect blood for evaluation of laboratory parameters (*Appendix E*):
  - Hematology panel
  - Full serum chemistry panel
  - Tumor lysis labs required just prior to C2D1 AZA dose and 2 hours post C2D1 AZA dose (*Appendix A-3*)

- Collect peripheral blood for PD analyses (*Section 7.4, Table 9 and Table 10*)
- Collect urine or serum sample for β-hCG pregnancy test for females of child-bearing potential
- Administer prophylactic antibiotics, antivirals, antifungals, antiemetics, antidiarrheals according to standard practices at each investigational site
- Administer DEC on Days 1-5 ***(during dose escalation)*** or AZA on 7-day or 5-2-2 schedule according to *Section 4.4.2.1*
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.2.3 Cycles 2+ – Day 8 (Patients receiving DEC + Alvocidib)
Cycles 2+ – Day 10 (Patients receiving AZA + Alvocidib)

- Abbreviated physical examination (AE- or symptom-directed exam)
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Assess ECOG PS (*Appendix B*)
- Administer prophylactic antibiotics, antivirals, antifungals, antiemetics, antidiarrheals according to standard practices at each investigational site
- Administer alvocidib according to *Section 4.4.2**.1***
- Collect blood for evaluation of laboratory parameters just prior to the start of the infusion and again approximately 4 hours post end of IVI (*Appendix E*):
    - Hematology panel
    - Full serum chemistry panel
    - Tumor lysis labs required just prior to C2D10 alvocidib IVI and 2 hours after end of alvocidib IVI in patients receiving AZA + alvocidib (*Appendix A-3*)
- Collect peripheral blood for PD analyses (*Section 7.4*, *Table 9* and *Table 10*)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.2.4- Cycles 2+ – Day 9 (Patients receiving DEC + Alvocidib)
Cycles 2+ – Day 11 (Patients receiving AZA + Alvocidib)

- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Collect blood for follow-up evaluation of laboratory parameters (*Appendix E*):
  - Hematology panel
  - Full serum chemistry panel

  After Cycle 3, frequency of labs may be performed as per standard of care starting after Day 9 for patients on DEC (during dose escalation) and after Day 11 for patients on AZA.
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.2.5 Cycles 2+ – Days 15, 22 (±3 Days)

Perform the following activities and evaluations on Days 15 and 22 of Cycles 2+:

- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Collect blood for follow-up evaluation of laboratory parameters (*Appendix E*):
  - Hematology panel
  - Full serum chemistry panel
- Collect peripheral blood on Day 15 for PD analyses (*Section 7.4, Table 9* and *Table 10*)
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.2.2.6 Cycles 2+ – Day 28 (+1/-3 Days)

Perform the following evaluations on Day 28 of every EVEN cycle (ie, Cycle 2, Cycle 4, etc):

- Perform bone marrow biopsy and aspiration and collect peripheral blood sample for assessment of response (*Appendix F*), standard cytogenetics, and PD analyses (*Section 7.4, Table 9* and *Table 10**;***

Appendix E). [Note with a +1/-3 day window, these may be performed on Days 25-28 or Day 1 of the next cycle. If performed on Day 1 of the next cycle, please collect bone marrow and aspiration as well as peripheral blood samples prior to administering first dose of DEC or AZA]

- If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, at the EVEN 2+ cycle visits, there should be no delay in dosing and the procedure should be repeated within 7 days.
- Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.

- Response assessments ***with*** standard cytogenetics ***and*** blood sample collection for PD analyses should be repeated on Day 28 (+1/-3 days) of Cycles 2, 4, and 6, and then every 4 cycles, thereafter, or as clinically indicated. If medically appropriate, response assessments should be repeated at the time of disease progression.
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.

5.3 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.

Perform the following assessments whenever a patient discontinues study treatment/is withdrawn:

- Perform a full physical examination including weight (kg) and other measures of disease and disease symptoms, eg, extramedullary disease
- Record vital signs (temperature, heart rate, systolic and diastolic blood pressures)
- Assess ECOG PS (*Appendix B*)
- Collect blood for follow-up evaluation of laboratory parameters (*Appendix E*):
  - Hematology panel
  - Full serum chemistry panel
  - Coagulation panel (as clinically indicated)

- If ≥8 weeks since last response assessment, perform bone marrow biopsy and aspiration and collect peripheral blood sample for assessment of response (*Appendix F*), and PD analyses (*Section 7.4, Table 9* and *Table 10**; **Appendix E***)
    - If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days.
    - Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.
- Collect urine or serum sample for β-hCG pregnancy test for females of child-bearing potential
- Assess for AEs
- Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE

5.4 FOLLOW-UP ASSESSMENTS

5.4.1 Follow Up for Safety

Patients must have a safety evaluation 30-45 days after the last dose of study drug (ie, 30 days +15-day window). This evaluation can be done by phone. The following assessments will be performed:

- Assess for AEs
    - Ongoing AEs must be followed clinically until they resolve, are deemed permanent or no longer clinically significant, or the patient begins an alternative treatment regimen
- 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.

5.4.2 Follow Up for Overall Survival

All patients enrolled and treated in the Phase 2 study will be contacted by telephone to assess for date of death, date of stem cell transplant, date of relapse, or continued remission for three years:

- Year 1: Phone calls monthly beginning the month after the patient completes the end-of-study assessments to 12 months after Date of First Dose regardless of how many cycles a patient receives (Year 1 = Date of First Dose plus 12 months)
- Year 2: Phone calls every other month during Year 2 (months 14 to 24 months after Date of First Dose).
- Year 3: Phone calls every three months during Year 3 (months 25 to 36 months after Date of First Dose).

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 (*Appendix A*), should be completed at the time of the withdrawal, wherever possible, including dates of response and death. If the study patient is prematurely withdrawn due to an AE, attempts should also be made to clinically follow the study patient until the event is resolved, stable or permanent as determined by the PI and Sponsor.

6.2 REASONS FOR WITHDRAWAL

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

- Failure to achieve a response. Patients not demonstrating evidence of CR/CRi/CRmarrow/PR/HI after the second cycle of treatment will be considered for removal from the study, although with permission of the Medical Monitor, induction treatment may continue if clinically indicated and provided there is no evidence of toxicity ≥NCI CTCAE grade 4;
- An excessive Grade 3-4 toxicity without a response to treatment or occurrence of any other AE, concurrent illness or laboratory abnormality which, in the opinion of the PI, warrants the patient's permanent withdrawal;
- Patient noncompliance, defined as refusal or inability to adhere to the study schedule;
- At the request of the patient, PI, the Sponsor, or regulatory authority;
- Patient is lost to follow-up;
- 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 AE must be followed clinically until the event is resolved, deemed stable or permanent, or the patient begins an alternative treatment regimen. A stable adverse event is defined as an event that is not expected to change in nature, severity or frequency. See *Sections 8.4 – 8.8* for reporting of adverse events. Patients withdrawn from the study for pregnancy will be followed according to *Section 5.4*. The pregnancy of any patient, or patient's partner, will be followed to term to record any birth defects/abnormalities at time of birth.

6.4 TERMINATION OR SUSPENSION OF STUDY

Should the Sponsor or their representatives, Investigators, or appropriate regulatory officials discover conditions during the study that indicate that the study or site involvement should be put 'On Hold' or 'Terminated', this action may be taken after appropriate consultation with the Sponsor, Investigators and Study Monitors.

Conditions that may warrant termination of the study or involvement of a study site include, but are not limited to:

- If the Data Safety Monitoring Board (DSMB)* recommends stopping the study due to safety concerns (*if applicable, as DSMBs may not be utilized in every study);
- The decision on the part of the Sponsor to suspend or discontinue testing, evaluation, or development of the study drug
- The discovery of an unexpected, serious, unacceptable risk to patients enrolled in the study;
- Failure of the Investigator(s) to comply with pertinent clinical trial regulations; or
- Submission of knowingly false information from the research facility to the Sponsor, study monitor, or regulatory agencies.
- Insufficient adherence to protocol requirements.

Study termination and follow-up will be performed in compliance with the conditions set forth in the International Council on Harmonisation (ICH) sixth efficacy publication (E6) on Good Clinical Practice (GCP), Section 4.12, ICH E6 4.13, ICH E6 5.20, and ICH E6 5.21, with appropriate notification of the Food and Drug Administration (FDA) and all IRBs having approved the study protocol and ICF.

7. CRITERIA FOR EVALUATION

7.1 SAFETY ENDPOINTS

Safety and tolerability of alvocidib when administered in sequence after DEC ***(during dose escalation)*** or AZA will be assessed by analyzing the DLTs, MTD, and incidence rates of treatment-emergent adverse events (TEAEs) summarized within treatment group(s) 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, physical examinations, vital sign measurements, and clinical laboratory testing [hematology, serum chemistry, and coagulation]) will be analyzed as additional measures of safety and tolerability for the entire study duration. These assessments will be summarized by shift tables and treatment group using mean, standard deviation, median, minimum and maximum changes from baseline values.

Mortality (all causes) will be calculated.

7.2 EFFICACY ENDPOINTS

Primary Efficacy Endpoints

- Assess preliminary efficacy, as determined by response rate, duration of response, hematological improvement, rate of transfusion dependence, time to AML, and overall survival.

Efficacy endpoints will be any objective response to study drug therapy using assessments defined by the 2006 revised International Working Group (IWG) criteria [28] and European MDS Working Group.

Secondary Efficacy Endpoints

- The proportion of MDS patients in the overall population achieving RBC transfusion independence with duration ≥84 days (12 weeks) during treatment. Baseline RBC transfusion dependence is defined as an average transfusion requirement of ≥2 units/28 days of RBCs without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose. Transfusion data include the hemoglobin value for which the transfusion was administered and must have been ≤10.0 g/dL within 7 days prior to transfusion administration to be counted toward the RBC transfusion-dependent status.

- Platelet transfusion independence with duration ≥84 days (12 weeks) will be assessed in the proportion of patients who were platelet transfusion dependent at baseline. Baseline platelet transfusion requirements are established by the number of platelet transfusions administered during the 56 days immediately preceding and including the date of first dose.

- Erythroid hematologic improvement and platelet hematologic improvement will be assessed according to International Working Group 2006 criteria.

- Correlation of complete response rates with BH3 profiling results including MCL-1 dependency

- Overall survival compared to historical data

Complete details of the planned analysis will be documented in a full Statistical Analysis Plan (SAP), which will be finalized before locking the study database.

7.3 PHARMACOKINETIC ENDPOINTS

Plasma concentrations of alvocidib will be summarized by descriptive statistics, including mean, n, standard deviation, coefficient of variation, minimum, maximum, and median. Prior to analysis of study samples, the assay sensitivity, specificity, linearity, and reproducibility will be documented.

Parameters to be assessed include the time to C_{max} (Peak time, T_{max}) and the area under the plasma concentration versus time curve (AUC) from time 0 to 24 hours post-dose (AUC_{0-24}), the maximum observed plasma concentration (C_{max}), half life ($t_{1/2}$), AUC from 0 to time t (AUC_t), AUC from time 0 to infinity (AUC_{0-inf}), and clearance (CL).

Plasma PK analyses for alvocidib and known metabolites, if any, and dose proportionality will be determined during Cycle 1 on Days 8 and 9 (in patients receiving alvocidib following DEC) or Days 10 and 11 (in patients receiving alvocidib following AZA) (*Table 8*).

Table 8: Timing of PK Sampling during Cycle 1 of Phase 1b Study

PK Sample No.	Day (Regimen)	Timepoint in Relation to Alvocidib Administration	Window
1	D8 (DEC + ALV) / D10 (AZA + ALV)	Prior to IVI	At any time
2		End of IVI	+10 mins
Up to 30-min wait (± 10 mins) [a]			
3	D8 (DEC + ALV) / D10 (AZA + ALV)	30 mins after end of IVI	±10 mins
4		1 hr after end of IVI	±10 mins
5		2 hrs after end of IVI	±15 mins
6		4 hrs after end of IVI	±15 mins
7	D9 (DEC + ALV) / D11 (AZA + ALV)	After alvocidib IVI (23 hrs after start of IVI in pts receiving hybrid dosing or ***start*** of infusion in pts receiving ***30-60 min*** IVI)	±1 hr

ALV = alvocidib; AZA = azacitidine; DEC = decitabine; IVI = IV infusion
a This wait time is only required in patients receiving DEC following alvocidib hybrid dosing (ie, 30-min IV bolus; up to 30-min wait; and then the 4-hr IVI).

7.4 PHARMACODYNAMIC ENDPOINTS

The pharmacodynamic relationship between alvocidib exposure and exploratory biomarkers will be quantified, specifically looking at the correlation between the rate of response (CR/CRi/CRmarrow/PR/HI) and BH3 profiling by flow cytometry with an emphasis on MCL-1 dependence.

The types of biomarkers to be analyzed may include, but are not limited to, nucleic acids, proteins, lipids or metabolites. Biomarker assessments may be used to assess and generate prognostic, predictive pharmacodynamics or surrogate biomarker signatures. These assessments may be explored in the context of MDS or related conditions or drugs of similar class. The results from these analyses are exploratory in nature and may not be included in a clinical study report (CSR).

Collection of peripheral blood samples for PD analyses will follow the schedules outlined in *Table 9* (DEC plus alvocidib) and *Table 10* (AZA plus alvocidib) ***(Appendix E)***.

Table 9: Timing of PD Sampling during Phase 1b and Phase 2 (Patients Receiving DEC + ALV)

PD Sample No.	Cycle/Day	Timepoint in Relation to Drug Administration	Window
1	Screening	Day -28 to Day 1	At any time
2	C1D1	Prior to DEC infusion	≤10 mins prior
3	C1D3	Prior to DEC infusion	≤10 mins prior
4	C1D5	Prior to DEC infusion	≤10 mins prior
5	C1D8	Prior to alvocidib IVI	At any time
6	C1D9	After alvocidib IVI (23 hrs after start of 4-hr IVI)	Within ±1 hr
7	C1D15	During Study Visit	±3 days
8	C1D22	During Study Visit	±3 days
9	C2D1	Prior to DEC infusion	At any time
10	C2D8	Prior to alvocidib IVI	At any time
11	C2D15	During Study Visit	±3 days
12	C2D28	During Study Visit	+1 / -3 days
13	C4* + D28	During Study Visit	At any time
14	EOS	During Study Visit	At any time

* Even cycles 4 ***and 6 and every 4 cycles thereafter (ie, Cycle 10, Cycle 14, etc).***
DEC = decitabine; EOS = end of study; IVI = IV infusion

Table 10: Timing of PD Sampling during Phase 1b and Phase 2 (Patients Receiving AZA + ALV)

PD Sample No.	Visit/Day	Timepoint in Relation to Drug Administration	Window
1	Screening	Day -28 to Day 1	At any time
2	C1D1	Prior to AZA infusion	≤10 mins prior
3	C1D3	Prior to AZA infusion	≤10 mins prior
4	C1D5	Prior to AZA infusion	≤10 mins prior
5	C1D7	Prior to AZA infusion	≤10 mins prior
6	C1D10	Prior to 30-min alvocidib IVI	At any time
7	C1D11	After alvocidib IVI (23 hrs after ***start*** of ***30-60 min*** IVI)	Within ±1 hr
8	C1D15	During Study Visit	±3 days
9	C1D22	During Study Visit	±3 days
10	C2D1	Prior to AZA infusion	At any time
11	C2D10	Prior to alvocidib IVI	At any time
12	C2D15	During Study Visit	±3 days
13	C2D28	During Study Visit	+1 / -3 days
14	C4* + D28	During Study Visit	At any time
15	EOS	During Study Visit	At any time

* Even cycles 4 ***and 6 and every 4 cycles thereafter (ie, Cycle 10, Cycle 14, etc).***
AZA = azacitidine; EOS = end of study; IVI = IV infusion

The samples may be retained for no longer than 20 years after study completion or per local requirements.

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 Investigational New Drug (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 v5.0 (*see Appendix D*). 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)
- Possibly: AE *may be related* to the investigational agent(s)
- Probably: AE is *likely related* to the investigational agent(s)
- Definitely: 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 seen as part of the spectrum of underlying MDS and are expected events during MDS therapy. Therefore, myelosuppression and associated complications directly related to the myelosuppression, such as fever, febrile neutropenia, infections, anemia, thrombocytopenia, and bleeding, will not be reported as serious adverse events (SAEs) but will be reported as AEs on the AE CRF and will be summarized in the updated and final reports.

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 PI 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.

A laboratory abnormality will be reported on the "Adverse Event" case report form only if it is the main causative factor of an SAE, requires a therapeutic intervention, is the reason for a patient coming off study, or meets DLT criteria. Record the highest grade of the laboratory abnormality with start and stop dates associated with that grade. Laboratory values for hematology and serum chemistry parameters will be collected during the time the patient is on study to facilitate duration of worst CTCAE grade per cycle change-from-baseline analyses and overall worst CTCAE grade during the study. Any laboratory parameters assessed outside the per-protocol visits (ie, interim labs) will be recorded as 'Unscheduled Laboratory Assessments' in the database.

Adverse events will be reported and described in terms of intensity, seriousness and causality, based on the PI'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 PI's awareness of the event, whether or not this reaction is considered to be associated with use of the investigational drug. In addition, the occurrence of any AE leading to permanent discontinuation of study drug must also be reported to the Sponsor within 24 hours of the PI's awareness of the event.

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

Email: CRF@couranteoncology.com

It is expected that the PI will provide or arrange appropriate supportive care for the study patient. A patient experiencing a SAE should be followed clinically until the event resolves, is deemed permanent or no longer clinically significant, or the patient begins an alternative treatment regimen. All telephone and scanned/emailed reports must be followed with a written Serious Adverse Event (SAE) report form within 24 hours of the PI'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 PI, 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 AEs or new AEs identified on the last scheduled visit should be followed by the PI until the AEs resolve, are deemed permanent or no longer clinically significant, or the patient begins an alternative treatment regimen. Resolution means the patient has returned to his/her baseline state of health or the PI does not expect any further improvement or worsening of the adverse event. The PI 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 PI which occur after the last scheduled visit, and are determined by the PI 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 AE or SAE must be followed clinically until the event is resolved, deemed permanent or no longer clinically significant, or the patient begins an alternative treatment regimen. A stable adverse event is defined as an event, which is not expected to change in nature, severity, or frequency. The PI 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 Food and Drug Administration (FDA) as described in 21 CRF Section 312.32 (IND Safety Reports) and to other Regulatory Authorities according to local regulations.

In addition, the PI is required by FDA regulations to notify the Institutional Review Board (IRB) promptly of all unexpected SAEs occurring at the investigator's study site. The PI 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 DRUGS

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

Alvocidib is supplied for parenteral administration as a sterile, nonpyrogenic, injectable, clear pale yellow to yellow-colored, 10 mg/mL solution, which is packaged in glass vials fitted with coated rubber closures crimped with an aluminum seal and 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).

Decitabine and azacitidine are approved pharmaceutical products and will be provided by commercially available sources.

Decitabine for Injection is a white to almost-white sterile, lyophilized powder supplied in a clear colorless glass vial. Each 20-mL, single-dose, glass vial contains 50 mg decitabine, 68 mg monobasic potassium phosphate (potassium dihydrogen phosphate) and 11.6 mg sodium hydroxide sterile [29].

Azacitidine for injection is a white to off-white solid. Each 100-mg, single-dose vial contains 100 mg of azacitidine and 100 mg mannitol as a sterile lyophilized powder [30].

9.2 STUDY DRUG DISPENSING AND ACCOUNTABILITY

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

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

drug vials should be returned to the Sponsor or ***the Contract Research Organization (CRO) and/or drug may be authorized for destruction onsite*** 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, United States Pharmacopeia (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 4.4.2.1*.

Decitabine should be aseptically reconstituted with 10 mL of Sterile Water for Injection, USP; upon reconstitution, each mL contains approximately 5.0 mg of decitabine at pH 6.7-7.3 [29]. Immediately after reconstitution, the solution should be further diluted with 0.9% Sodium Chloride Injection or 5% Dextrose Injection to a final drug concentration of 0.1-1.0 mg/mL. Unless used within 15 minutes of reconstitution, the diluted solution must be prepared using cold (2°C to 8°C) infusion fluids and stored at 2°C to 8°C (36°F to 46°F) for up to a maximum of 4 hours until administration. The diluted solution should be administered according to treatment schedule provided in *Section 4.4.2.1.*

Azacitidine should be aseptically reconstituted with Sterile Water for Injection, USP, per the US prescribing information and based on intended route of administration [30]. (Azacitidine is incompatible with 5% Dextrose solutions, Hespan, or solutions that contain bicarbonate. These solutions have the potential to increase the rate of degradation of azacitidine and should therefore be avoided). The total dose should be administered according to treatment schedule provided in *Section 4.4.2.1*. Administration must be completed within 1 hour of reconstitution of the vial(s).

Decitabine and azacitidine 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 between 2°C to 30°C (36°F to 86°F)
- Decitabine should be stored at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F)
- Azacitidine unreconstituted vials should be stored at 25°C (77°F); excursions permitted to 15°C to 30°C (59°F to 86°F)

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 CRFs) of all subjects' visits, being sure to include all pertinent study related information. A statement should be made indicating that the subjects have been enrolled in this clinical study and have provided written Informed Consent. Any adverse events must be thoroughly documented. Results of any diagnostic tests conducted during the study should also be included in the source documentation.

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

The PI 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, institutional review board/independent ethics committee [IRB/IEC] approval) and pertinent hospital or clinic records for confirmation of data throughout the study period.

10.2 SOURCE DOCUMENT MAINTENANCE

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

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

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

Source documents can include, but are not limited to:

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

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

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

10.3 RECORD MAINTENANCE

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

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

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

The PI 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 (DCFs) 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 PI 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, current version, and 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 PI and Subinvestigators.
- Financial Disclosure Form of PI and Subinvestigators (if applicable).

- Department of Health and Human Services Number for IRB, or other documentation of IRB compliance with FDA regulation (United States 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 PI, 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.
- Clinical Research Associate (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 CONSIDERATIONS

11.1 GENERAL CONSIDERATIONS

Protocol TPI-AVL-102 is a Phase 1b / 2, multicenter, open-label study in which Phase 1b will employ the 3+3 dose-escalation design, dose expansion at MTD/RP2D, and Phase 2 utilizing the Simon 2-stage minimax design [27].

Results of statistical analyses, descriptive statistics, and supporting listings will be presented by study phase (Phase 1b and Phase 2) and within alvocidib dose level. In addition, overall statistics will be calculated for Phase 1b patients across all alvocidib dose levels and for the total study population (ie, regardless of study phase).

Statistical analysis for all safety and efficacy parameters will be primarily descriptive in nature. Categorical variables will be summarized by frequency distributions (number and percentages of patients), continuous variables will be summarized by mean, standard deviation, median, minimum, maximum, and time-to-event variables will be summarized using Kaplan-Meier methods and figures for the estimated median time. No formal statistical hypothesis testing is planned; however, if exploratory analyses are conducted and confidence intervals (CIs) are provided for estimates, the 95% confidence intervals are consistent with a 2-sided 5% significance level. All analyses, summaries, and listings will be performed using SAS software (version 9.4 or higher)

A detailed methodology for summary and statistical analysis of the data collected in this study will be documented in a Statistical Analysis Plan (SAP) that will be finalized prior to database lock. The SAP may modify the data analysis plans outlined in the protocol; and if so, will be clearly documented in the SAP. Any major modifications of the study design or study endpoints and/or its analysis will also be reflected in a protocol amendment.

11.2 SAMPLE SIZE CONSIDERATIONS

Phase 1b

Patients will be enrolled in cohorts of 3-6 patients. Escalation of the alvocidib dose will follow a standard 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 (*Section 4.5.3*). Per the standard oncology 3+3 Phase 1 dose escalation design, the total number of patients to be enrolled cannot be precisely determined as the sample size is dependent upon the observed safety profile, which will determine the number of patients per dose cohort, as well as the number of dose escalations required to achieve the MTD. It is anticipated that a maximum of 24 patients will be required to reach MTD and determine the RP2D.

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. Adverse events meeting the definition of DLT during Cycles 2+ will be taken into consideration when evaluating dose escalation.

Once the MTD or preliminary RP2D of alvocidib administered via hybrid dosing (ie, IV bolus followed by IVI) is identified, 2 cohorts of at least 3 patients each will receive AZA followed in sequence by alvocidib with the total dose of alvocidib administered by ***30- to 60-minute*** IVI.

Expansion at MTD

Once the MTD or preliminary RP2D of alvocidib administered by ***30- to 60-minute*** IVI is determined, up to 25 patients will be enrolled in an Expansion cohort to receive alvocidib following either DEC or AZA to confirm safety, explore potential biomarkers, and evaluate potential signals of alvocidib activity. Once this Expansion cohort is completed, the study will progress to Phase 2.

Phase 2

Stopping Rules based on Efficacy

The statistical power calculations for Phase 2 are based on the Simon 2-stage minimax design [27].

- Stage 1: Up to 15 evaluable patients will be enrolled and treated at the RP2D identified in the Phase 1b study. Stage 2 may be initiated at any point after confirming a response (CR/CRi/CRmarrow/PR/HI) in two Stage 1 patients. If there is ≤1 responder among 15 evaluable Stage-1 patients, the study will be stopped after Stage 1.

- Stage 2: Ten patients will be enrolled to bring the total enrollment in Phase 2 (including Stage-1 patients) to 25 evaluable patients. Stage-2 patients will also receive the RP2D dose of alvocidib administered by ***30- to 60-minute*** IVI identified in the Phase 1b study. If 6 or more responses are observed in 25 patients, the conclusion will be that the combination regimen is worthy of further investigation. When the true response rate of 30% (alternative hypothesis) is tested against the null hypothesis response rate of 10%; this design yields a Type I error rate of 0.05 and power of 80%.

Any patient who withdraws from Stage 1 or 2 for treatment-related toxicity or disease progression or dies prior to being evaluated for response, they will be considered a non-responder. Patients who drop out for other reasons prior to being assessed for response will be considered unevaluable and may be

replaced. Enrollment into Phase 2 may be stopped at any point once ≥6 patients have had a response to treatment, but the maximum enrollment in Phase 2 will be 25 evaluable patients.

Stopping Rules based on Safety

The study may be stopped early during Phase 2 in the event of unacceptable toxicities. Early stopping rules for safety are based on a Sequential Probability Ratio Test (SPRT) with a baseline toxicity rate of 5%; an upper ceiling of unacceptable toxicity of 20% (ie, toxicities meeting one or more DLT criteria as stated in *Section 4.5.3*); and alpha=0.05 with power=80%.

The study will be stopped if unacceptable toxicities are observed in:

- 2 of the first 2 patients
- 3 of the first 11 patients
- 4 of the first 20 patients
- 5 of the first 29 patients (Ph 2 study to enroll maximum of 25 patients, but considering possible replacement patients)

11.3 RANDOMIZATION AND BLINDING

This is an open-label, single arm, Phase 1b / 2 study; thus, randomization and blinding are not part of the study design.

11.4 ANALYSIS POPULATION SETS

This study will have three analysis populations:

- **Intent-to-Treat (ITT)** analysis set includes all patients who were enrolled into the study.
- **Safety Analysis Set** consists of all patients who received study treatment (alvocidib).
- **Response Evaluable Set** consists of patients who have at least one post-baseline efficacy assessment; patients without a post-baseline efficacy assessment will not be considered evaluable for the primary efficacy analysis. Patients who discontinue due to disease progression or die or treatment-related toxicity prior to having a disease assessment will be included in the Response Evaluable population.

11.5 PATIENT DISPOSITION

A detailed description of patient disposition will be provided. It will include:

- The number of patients who were enrolled, included in the ITT, safety, and response evaluable analysis sets.
- A summary of patient dose cohorts.
- A summary of patients who complete the protocol.
- A summary of reasons for withdrawal from study.
- A summary of reasons for patients with treatment failure.
- An account of all identified protocol violations.
- All patients enrolled in the study will be accounted for in the summation.

11.6 DEMOGRAPHIC AND BASELINE CHARACTERISTICS

Demographic characteristics including age, gender, race, and ethnicity will be presented in the form of tabular summary statistics. Other subject baseline characteristics including but not limited to: weight, height, body surface area, initial stage of disease, prior therapies, and ECOG performance status will be presented similarly.

11.7 CONCOMITANT MEDICATIONS

The number and proportion of patients using different concomitant medications will be tabulated and summarized by WHODrug anatomical therapeutic chemical (ATC) and preferred term.

11.8 TREATMENT ADMINISTRATION / COMPLIANCE

Study drug administration will be described in terms of the total number of cycles administered, the median (range) of cycles administered, dose intensity, and reasons for the deviations from planned therapy.

11.9 EFFICACY ANALYSIS

The efficacy endpoints include the following:

- Complete Response Rate (CRR) is the percentage of patients achieving a CR, CRi, CRmarrow, PR, or HI. Patients who, for any reason, do not supply bone marrow for response assessment are counted among those not achieving CR/CRi/CRmarrow/PR/HI. CRR will be summarized by number and percentage of patients meeting the definition of CRR along with the corresponding exact 95% confidence intervals.

- Duration of response and time to AML will be summarized by Kaplan-Meier methods (median, 95% CI, number of events, number censored, and Kaplan-Meier figures),

- Hematological improvement and rate of transfusion independence will be summarized using the appropriate descriptive statistics and 95% confidence intervals.

- Overall survival (OS), defined as the time from first dose to death from any cause. OS will be summarized by Kaplan-Meier methods (median, 95% CI, number of events, number censored, and Kaplan-Meier figures).

Descriptive statistics will also be calculated for each secondary measure of efficacy.

- The proportion of MDS patients in the overall population achieving RBC transfusion independence with duration ≥84 days (12 weeks) during treatment. Baseline RBC transfusion dependency is defined as an average transfusion requirement of ≥2 units/28 days of RBCs without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose. Transfusion data include the hemoglobin value for which the transfusion was administered and must have been ≤10.0 g/dL within 7 days prior to transfusion administration to be counted toward the RBC transfusion-dependent status.

- Platelet transfusion independence with duration ≥84 days (12 weeks) will be assessed in the proportion of patients who were platelet transfusion-dependent at baseline. Baseline platelet transfusion requirements are established by the number of platelet transfusions administered during the 56 days immediately preceding and including the date of first dose.

- Erythroid hematologic improvement and platelet hematologic improvement will be assessed according to the 2006 revised IWG criteria [28].

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

11.10 SAFETY ANALYSIS

Safety will be monitored during the period starting after receipt of the first dose of study drug and ending 30 days after the final administration of alvocidib. During Phase 1, the safety endpoints will be evaluated after Cycle 1. The dose escalation committee will have access to complete safety profiles of all patients receiving alvocidib and will be able to take decisions accordingly.

All patients who receive any dose (any amount) of alvocidib will be included in the summaries and listings of safety data. Overall safety profile will be characterized by type, frequency, severity, timing, duration and relationship of study drug of adverse events and laboratory abnormalities.

11.10.1 Adverse Events

Adverse events will be classified using the MedDRA classification system version 20.0 or higher. The severity of the toxicities will be graded according to the NCI CTCAE v5.0.

In all summaries, emphasis will be placed on TEAEs, namely, those with initial onset or that worsen in severity after the first dose of alvocidib. Adverse events will be summarized by the frequency of patients experiencing TEAEs corresponding to body systems and MedDRA preferred term and by worst NCI CTCAE (v5.0) grade. Summaries will also be provided of treatment-related TEAEs, namely, those judged by the investigator to be related or likely related to alvocidib and/or combination regimens.

Adverse events resulting in discontinuation of alvocidib treatment or withdrawal from the study, Grade 3 or higher, serious adverse events, and deaths on-study will be tabulated.

All DLTs will be reported and the MTD and RP2D identified.

11.10.2 Laboratory Tests

Laboratory data will be summarized for the observed values at each scheduled assessment, together with the corresponding changes from baseline (the value obtained prior to dosing on Day 1 of Cycle 1) using descriptive statistics.

For those analytes with NCI CTCAE v5.0 severity criteria are specified, abnormal laboratory values will be summarized by shift tables displaying numerical values and percentages classified by Baseline grade (ie, grade prior

to dosing on Day 1 of Cycle 1) and maximum grade on treatment. All laboratory data will be presented in listings.

11.10.3 Vital Signs and Physical Examination Findings

Vital signs data will be summarized by the observed valued at each scheduled assessment, together with the corresponding changes from baseline using descriptive statistics.

Physical examination findings will be presented in data listings.

11.11 INTERIM ANALYSIS

For Phase 1b, safety data will be monitored continuously per standard Phase 1 oncology study practices.

For Phase 2, because the Simon 2-stage minimax design will be employed, response rate data will be assessed after Stage 1 and Stage 2.

11.12 PHARMACOKINETIC ANALYSIS

Pharmacokinetic parameters will be estimated using standard noncompartmental methods and according to FDA guidance. PK parameters include:

- C_{max} = maximum observed plasma concentration
- T_{max} = time to C_{max} (peak time)
- AUC_{0-24} = area under the plasma concentration curve from time 0 to 24 hours
- AUC_{0-inf} = AUC from time 0 to infinity
- AUC_t = area under the plasma concentration curve from time 0 to time t
- $t_{1/2}$ = half life
- CL = clearance using noncompartmental methods

Actual sample collection times will be used rather than scheduled collection times. Plasma concentrations below the limit of quantification will be treated as 0. Imbedded missing plasma concentrations (ie, missing values between two observed values) will be estimated using linear extrapolation. This is consistent with using the trapezoidal rule to calculate AUC. Other missing plasma concentrations will be excluded from calculations to estimate PK parameters.

11.13 PHARMACODYNAMIC ANALYSIS

Peripheral blood and bone marrow samples will be collected at protocol-specific time points to assess the effects of alvocidib when administered in sequence after DEC ***(during dose escalation)*** or AZA. Analyses may include, but are not limited to: correlation of complete response rates with BH3 profiling by flow cytometry with an emphasis on MCL-1 dependence, evaluating recurrent genetic mutations, and other biomarkers associated with MDS.

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 PI must await IRB/IEC approval of the amendments before implementing the changes. However, a protocol change which is intended to eliminate an apparent immediate hazard to patients may be implemented immediately, provided the IRB/IEC is 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 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 ***Sumitomo Dainippon Pharma Oncology,*** Inc ***(SDP Oncology)***, in compliance with the applicable international and local regulatory requirements as well as applicable ICH guidelines, Declarations of Helsinki (1964, 1975, 1983, 1989, 1996, 2000, 2002, 2004, 2008, 2013) and in respect of the Sponsor or designee's standard operating procedures (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 findings will be provided by Quality Assurance, in writing.

15. ETHICS AND RESPONSIBILITY

15.1 PRINCIPAL INVESTIGATOR'S RESPONSIBILITIES

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

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

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

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

15.2 INFORMED CONSENT

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

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

15.3 INSTITUTIONAL REVIEW BOARD

This protocol and all amendments will be reviewed and approved by the Institutional or Independent Review Board(s) or Independent Ethics Committee(s) charged with this responsibility at the study center. Notification in writing of approval must come from the Chairman or the Secretary of the IRB/IEC meeting minutes where this protocol and associated informed consent form were discussed. The PI shall not participate in the decision, and, if an IRB/EC member, the written approval must indicate such non-participation. The PI shall submit status reports to the IRB/IEC no less frequently than annually (when applicable). The IRB/IEC must be notified by the PI in writing of the interruption and/or completion of the study; the PI must promptly report to the IRB/IEC all changes in research (protocol amendments) and will not make such changes without IRB/IEC approval, except where necessary to eliminate apparent immediate hazards to human patients. In these cases, the IRB/IEC must be notified within five days of the change. The PI will promptly report to the IRB/IEC all unanticipated problems involving risk to patients or others. The PI is required to maintain an accurate and complete record of all written correspondence to, and received from the IRB/IEC, and must agree to share all such documents and reports with the Sponsor.

16. CONFIDENTIALITY

The existence of this clinical study is confidential (with exception of listing on clinicaltrials.gov) 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 ***SDP Oncology***'s products or research programs that is provided by ***SDP Oncology*** 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 ***SDP Oncology***'s disclosure; (d) information which is lawfully disclosed to you by a third party not under any obligation of confidence to ***SDP Oncology***; 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 ***SDP Oncology***.

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 patient number in case report forms. 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 nonpatentable, shall be the sole property of ***SDP Oncology***, 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 ***SDP Oncology,*** Inc, the right to review all manuscripts, abstracts, and presentations related to this study *prior* to their submission for publication or presentation. ***SDP Oncology*** may use these data now and in the future for presentation or publication at ***SDP Oncology***'s discretion or for submission to government regulatory agencies.

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

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APPENDIX A-1 – SCHEDULE OF ACTIVITIES (Patients Receiving DEC and Alvocidib)
Phase 1b, Expansion, and Phase 2

Cycle	Predose		Cycle 1					Cycles 2+						End of Study [a]	Follow Up [b]
Day	–28 D	–72 Hr	D1	D8	D9	D15 (±3d)	D22 (±3d)	D1	D8	D9	D15 (±3d)	D22(±3d)	D28 (+1/-3d)		
Procedures/Tests															
Signed informed consent [c]	X														
Medical / disease history [d]	X														
Complete physical exam	X	X												X	
Height (cm)	X														
Weight (kg)	X	X						X						X	
Transfusion dependency [u]	X														
Vital signs [e]	X	X	X[q]	X	X	X	X	X[q]	X	X	X	X		X	
ECOG PS	X	X		X		X	X	X	X					X	
12-lead ECG plus QTc	X														
Chest radiograph ('x-ray') [f]	X														
CBC w/ manual diff & plts	X	X	X	X[v]	X	X	X	X	X[v]	X [gg]	X	X		X	
Serum chemistries [g]	X	X	X	X[v]	X	X	X	X	X[v]	X [gg]	X	X		X	
Coag panel: PT & aPTT	X													X [bb]	
Interim labs [g]	Laboratory assessments conducted at times other than per-protocol visits														
Pregnancy test [h]	X	X	X[s]					X						X	
BM biopsy/aspirate	X[n]												X[z]	X [cc]	
PD blood sampling	X[n]		X[r,w]	X[w]	X[w]	X[w]	X[w]	X[w]	X[w]		X[w]		X[z,aa]	X [cc]	
Concomitant medications [i]	X[o]	X[p]	X[t]	X[t]	X[t]	X[t]	X[t]	X[t]	X[t]	X[t]	X[t]	X[t]	X[t]	X[t]	X[t, dd]
Calculate BSA		X						X							
Baseline signs / symptoms		X	X[q]												
Confirm eligibility [j]		X													
Standard TLS prophylaxis		X [bb]	X [bb]	X [bb]	X [bb]			X [bb]	X [bb]	X [bb]					
Administer prophylactic medications [k]			X	X				X	X						
Decitabine administration (1-hr IVI on Days 1-5)			X					X							
Assessment of AEs [l]			X	X	X	X	X	X	X	X	X	X	X	X	X[ee]
Abbreviated physical exam[m]				X	X	X	X	X	X						
Alvocidib administration [ff]				X					X						
PK blood sampling				X[x]	X[y]										

Notes:

a. 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.
b. Patients must have a safety evaluation 30-45 days after the last dose of study drug (ie, 30 days +15-day window). This evaluation can be done by phone.
c. Written informed consent must be obtained prior to conduct of study-related screening evaluations. Evaluations performed as standard-of-care prior to obtaining consent may be utilized for screening.
d. Collect and document a complete medical and disease history including histologically confirmed diagnosis of MDS.
e. Vital signs to include: temperature, heart rate, systolic and diastolic blood pressures.
f. May omit if performed within 28 days prior to anticipated first dose.
g. Full serum chemistry panel to include: blood urea nitrogen, phosphorus, magnesium, lactate dehydrogenase, creatinine, uric acid, total protein, albumin, calcium, glucose, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and electrolytes (sodium, potassium, chloride, CO_2) (see *Appendix E*). All laboratory values for hematology and serum chemistry parameters collected during the time the patient is on study to facilitate change-from-baseline analyses. Any laboratory parameters assessed outside the per-protocol visits (ie, interim labs) will be recorded as 'Unscheduled Laboratory Assessments' in the database.
h. Collect urine or serum sample for β-hCG pregnancy test in females of child-bearing potential.
i. Including all prescription and nonprescription medications and nutritional supplements.
j. Review all inclusion and exclusion criteria to determine if patient has met all eligibility criteria for enrollment into the study. Obtain Medical Monitor (or designee) approval to enroll patient.
k. Including prophylactic antibiotics, antivirals, antifungals, antiemetics, antidiarrheals according to standard practices at each site.
l. Toxicities will be assessed according to the NCI CTCAE v5.0 (see *Appendix D*). When the NCI CTCAE grade is not available, the investigator will use the following toxicity grading: mild, moderate, severe, life-threatening, or fatal.
m. AE- or symptom-directed physical examination.
n. Perform bone marrow biopsy and/or aspiration and collect peripheral blood for disease status, standard cytogenetics, and PD analyses ***(Appendix E)***. If the initial bone marrow aspirate is nonproductive or not diagnostic, the procedure must be repeated. Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.
o. Including all prescription and nonprescription medications and nutritional supplements taken over previous 28 days.
p. Including all prescription and nonprescription medications and nutritional supplements taken since previous predose assessment visit.
q. Conducted just prior to first dose.
r. Collect peripheral blood samples for PD analyses on Days 1, 3, and 5 of Cycle 1 within 10 minutes prior to decitabine infusion (*Section 7.4, Table 9; **Appendix E***)
s. Required if screening pregnancy test was performed >72 hours prior to first dose.
t. Record only those medications (prescription, nonprescription, nutritional supplements) administered in conjunction with an AE.
u. Collect and document transfusion dependency, ie, transfusion requirement without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose and/or hemoglobin level. ***Record all transfusions (hemoglobin and platelet) for the 8 weeks/56 days prior to screening (including the transfusion date and number of units with each transfusion). Record all CBC data for the 8 weeks/56 days prior to screening including each CBC that prompted a transfusion.***
v. Collect blood just prior to start of infusion and again approximately 4 hrs post end of IVI (Time 0 = start of alvocidib IVI).
w. Collect peripheral blood for PD analyses (see *Section 7.4, Table 9; **Appendix E***).

x. **Phase 1b only** - Collect blood samples for PK analyses on Day 8 just before the alvocidib IVI (Sample #1) and at the end of the alvocidib IVI (Sample #2). The time between the end of the 30-min bolus and beginning of the 4-hr alvocidib IVI should be up to 30 mins. Continue to collect blood samples for PK analyses 30 mins after end of the alvocidib IVI (Sample #3), 1 hr after end of the alvocidib IVI (Sample #4), 2 hrs after end of the alvocidib IVI (Sample #5), and 4 hrs after end of the alvocidib IVI (Sample #6) (*Section 7.3, Table 8*).

y. **Phase 1b only** - Collect a blood sample for PK analysis from all patients on Day 9 at 23 hrs after ***the start*** of the alvocidib IVI administered on Day 8 (Sample #7) (*Section 7.3, Table 8*).

z. Perform bone marrow biopsy and aspiration and collect peripheral blood sample for assessment of response (*Appendix F*), standard cytogenetics, and PD analyses ***(Appendix E)***. [Note: with a +1/-3 day window, these assessments may be performed on Days 25-28 or Day 1 of the next cycle. If performed on Day 1 of the next cycle, collect bone marrow and aspiration as well as peripheral blood samples prior to administering the first dose of decitabine.]
If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, at the EVEN 2+ cycle visits, there should be no delay in dosing and the procedure should be repeated within 7 days. Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.
Response assessments and standard cytogenetics should be repeated on Day 28 (+1/-3 days) of Cycles 2, 4, and 6, and then every 4 cycles, thereafter, or as clinically indicated. If medically appropriate, response assessments should be repeated at the time of disease progression.

aa Peripheral blood samples to be collected for PD analyses on Day 28 of even cycles ***2,*** 4 ***and 6 and then every 4 cycles thereafter (ie, Cycle 10, Cycle 14, etc) or as clinically indicated (Appendix E).***

bb As clinically indicated.

cc If ≥8 weeks since last response assessment, perform bone marrow biopsy and aspiration and collect peripheral blood sample for assessment of response (*Appendix F*), and PD analyses ***(Appendix E)***. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days. Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.

dd Including any antineoplastic therapies initiated since discontinuation of study drug.

ee Ongoing AEs must be followed clinically until the event is resolved, deemed permanent or no longer clinically significant, or the patient begins an alternative treatment regimen.

ff Alvocidib to be administered at the dosages noted in *Table 5* first as a (30-min [±10 mins]) IV bolus followed up to 30 mins later by a 4-hr [±15 mins) IVI.

gg After Cycle 3, frequency of labs may be performed as per standard of care starting after Day 9.

APPENDIX A-2 – SCHEDULE OF ACTIVITIES (Patients Receiving AZA and Alvocidib)
Phase 1b, Expansion, and Phase 2

CYCLE	PREDOSE		CYCLE 1					CYCLES 2+						END OF STUDY [a]	FOLLOW UP [b]
DAY	–28 D	–72 HR	D1	D10	*D11*	D15 (±3d)	D22 (±3d)	D1	D10	*D11*	D15 (±3d)	D22 (±3d)	D28 (+1/-3d)		
PROCEDURES/TESTS															
Signed informed consent [c]	X														
Medical / disease history [d]	X														
Complete physical exam	X	X												X	
Height (cm)	X														
Weight (kg)	X	X						X						X	
Transfusion dependency [e]	X														
Vital signs [f]	X	X	X [u]	X [aa]	X	X	X	X [u]	X [aa]	X	X	X		X	
ECOG PS	X	X		X		X	X	X	X					X	
12-lead ECG plus QTc	X														
Chest radiograph ('x-ray') [g]	X														
CBC w/ manual diff & plts	X	X	X	X [z]	X	X	X	X	X [z]	X [jj]	X	X		X	
Serum chemistries [h]	X	X	X	X [z]	X	X	X	X	X [z]	X [jj]	X	X		X	
Coag panel: PT & aPTT	X													X [ff]	
Interim labs [i]	Laboratory assessments conducted at times other than per-protocol visits														
Pregnancy test [j]	X	X	X [v]					X						X	
BM biopsy/aspirate	X [r]												X [dd]	X [gg]	
PD blood sampling	X [r]		X [w,x]	***X*** [x]	***X*** [x]	X [x]	X [x]	X [x]	***X*** [x]		X [x]		X [dd,ee]	X [gg]	
Concomitant medications [k]	X [s]	X [t]	X [y]	X [y]	X [y]	X [y]	X [y]	X [y]	X [y]	X [y]	X [y]	X [y]	X [y]	X [y]	X [y,hh]
Calculate BSA		X						X							
Baseline signs / symptoms		X	X [u]												
Confirm eligibility [l]		X													
Administer prophylactic medications [m]			X	X				X	X						
TLS prophylaxis prior to AZA	See *Appendix A-3*														
TLS prophylaxis prior to alvocidib	See *Appendix A-3*														
Assessment of AEs [n]			X	X	X	X	X	X	X	X	X	X	X	X	X [ii]
AZA dosing (7-day or 5-2-2 schedule) [o]			X					X							
Abbreviated physical exam [p]				X	X	X	X	X	X						
PK blood sampling				***X*** [bb,cc]	***X*** [bb,cc]										
Alvocidib administration [q]				***X***					***X***						

Notes:

a 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.

b Patients must have a safety evaluation 30-45 days after the last dose of study drug (ie, 30 days plus a 15-day window). This evaluation can be done by phone.

c Written informed consent must be obtained prior to conduct of study-related screening evaluations. Evaluations performed as standard-of-care prior to obtaining consent may be utilized for screening.

d Collect and document a complete medical and disease history including histologically confirmed diagnosis of MDS.

e Collect and document transfusion dependency, ie, transfusion requirement without any consecutive 28-day gaps between transfusions for a minimum of 56 days immediately preceding first dose and/or hemoglobin level. ***Record all transfusions (hemoglobin and platelet) for the 8 weeks/56 days prior to screening (including the transfusion date and number of units with each transfusion). Record all CBC data for the 8 weeks/56 days prior to screening including each CBC that prompted a transfusion.***

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

g May omit if performed within 28 days prior to anticipated first dose.

h Full serum chemistry panel to include: blood urea nitrogen, phosphorus, magnesium, lactate dehydrogenase, creatinine, uric acid, total protein, albumin, calcium, glucose, total bilirubin, alkaline phosphatase, aspartate aminotransferase, alanine aminotransferase, and electrolytes (sodium, potassium, chloride, CO_2) (see *Appendix E*). All laboratory values for hematology and serum chemistry parameters collected during the time the patient is on study to facilitate change-from-baseline analyses.

i Any laboratory parameters assessed outside the per-protocol visits (ie, interim labs) will be recorded as 'Unscheduled Laboratory Assessments' in the database.

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

k Including all prescription and nonprescription medications and nutritional supplements.

l Review all inclusion and exclusion criteria to determine if patient has met all eligibility criteria for enrollment into the study. Obtain Medical Monitor (or designee) approval to enroll patient.

m Including prophylactic antibiotics, antivirals, antifungals, antiemetics, antidiarrheals according to standard practices at each site.

n Toxicities will be assessed according to the NCI CTCAE v5.0 (see *Appendix D*). When the NCI CTCAE grade is not available, the investigator will use the following toxicity grading: mild, moderate, severe, life-threatening, or fatal.

o Azacitidine (AZA) may be administered as either an IVI over 10 to 40 minutes or a subcutaneous (SC) injection on either a 7-day schedule (ie, Days 1-7 of AZA) or a 5-2-2 schedule (ie, Days 1-5 of AZA, Days 6-7 drug-free days, and Days 8-9 of AZA). (Schedule and route of AZA administration is at discretion of investigator).

p AE- or symptom-directed physical examination.

q Alvocidib to be administered by ***30- to 60-minute*** IVI according to the dose cohorts listed in *Table 6*.

r Perform bone marrow biopsy and/or aspiration and collect peripheral blood for disease status, standard cytogenetics, and PD analyses ***(Appendix E)***. If the initial bone marrow aspirate is nonproductive or not diagnostic, the procedure must be repeated. Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.

s Including all prescription and nonprescription medications and nutritional supplements taken over previous 28 days.

t Including all prescription and nonprescription medications and nutritional supplements taken since previous predose assessment visit.

u Conducted just prior to first AZA dose.

v Required if screening pregnancy test was performed >72 hours prior to first dose.

w Collect peripheral blood samples for PD analyses on Days 1, 3, 5, and 7 (Day 7 only in pts receiving 7-day AZA schedule) of Cycle 1 within 10 mins prior to AZA infusion (*Section 7.4, Table 10;* ***Appendix E***)

x Collect peripheral blood for PD analyses (see *Section 7.4, Table 10;* ***Appendix E***).

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

z Collect blood just prior to start of IVI and again approximately 4 hrs post end of IVI (Time 0 = start of ***30- to 60-minute*** alvocidib IVI).

aa Record vital signs (temperature, heart rate, systolic and diastolic blood pressures) 5-15 mins prior to initiation of alvocidib following a 5-min rest

bb Collect blood samples for PK analyses on Day 10 just before the alvocidib IVI (Sample #1) and at the end of the alvocidib IVI (Sample #2). Continue to collect blood samples for PK analyses 30 mins after end of the alvocidib IVI (Sample #3), 1 hr after end of the alvocidib IVI (Sample #4), 2 hrs after end of the alvocidib IVI (Sample #5), and 4 hrs after end of alvocidib IVI (Sample #6) (*Section 7.3*, *Table 8*).

cc Phase 1b only - Collect a blood sample for PK analysis from all patients on Day 11 at 23 hrs after ***start*** of the alvocidib IVI administered on Day 10 (Sample #7) (*Section 7.3, Table 8*).

dd Perform bone marrow biopsy and aspiration and collect peripheral blood sample for assessment of response (*Appendix F*), standard cytogenetics, and PD analyses ***(Appendix E)***. [Note: with a +1/-3 day window, these assessments may be performed on Days 25-28 or Day 1 of the next cycle. If performed on Day 1 of the next cycle, collect bone marrow and aspiration as well as peripheral blood samples prior to administering the first dose of AZA.]

If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, at the EVEN 2+ cycle visits, there should be no delay in dosing and the procedure should be repeated within 7 days. Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.

Response assessments and standard cytogenetics should be repeated on Day 28 (+1/-3 days) of Cycles 2, 4, and 6, and then every 4 cycles, thereafter, or as clinically indicated. If medically appropriate, response assessments should be repeated at the time of disease progression.

ee Peripheral blood samples to be collected for PD analyses on Day 28 of even ***C***ycle***s 2***, 4 ***and 6 and then every 4 cycles thereafter (ie, Cycle 10, Cycle 14, etc) or as clinically indicated (Appendix E)***.

ff As clinically indicated.

gg If ≥8 weeks since last response assessment, perform bone marrow biopsy and aspiration and collect peripheral blood sample for assessment of response (*Appendix F*), and PD analyses ***(Appendix E)***. If the bone marrow biopsy and/or aspirate is nonproductive or not diagnostic, the procedure must be repeated within 7 days. Six to 8 bone marrow slides will be prepared (in addition to fresh bone marrow samples) and sent to the Sponsor.

hh Including any antineoplastic therapies initiated since discontinuation of study drug.

ii Ongoing AEs must be followed clinically until the event is resolved, deemed permanent or no longer clinically significant, or the patient begins an alternative treatment regimen.

jj After Cycle 3, frequency of labs may be performed as per standard of care starting after Day 11.

APPENDIX A-3 – SCHEDULE FOR TLS EVALUATIONS (Patients Receiving AZA and Alvocidib) Phase 1b, Expansion, and Phase 2

Cycle	Cycle 1											Cycle 2		
Day	D1	D2	D3	D7	D8	D9	D10	D11	D12	D15 (±3d)	D22 (±3d)	D1	D10	Cycles 3+
Procedures/Tests														
Oral allopurinol	X [c]	X [c]	X [c]	X [c]	X [c]	X [c]	X [c]	X [c]	X [c]			X [o]		
IV hydration	X [d]						X [j]					X [d]		X [d]
Oral phosphate binder	X [e]	X [e]	X [e]	X [e]	X [e]	X [e]	X [k]	X [k]	X [k]			X [p]		
Tumor lysis labs [a]	X [f]	X [i]	X [i]				X [l]	X [n]	X [n]	X [n]	X [n]	X [r]	X [s]	X [t]
Monitor potassium levels	X [g,h]	X [g,h]	X [g,h]				X [m]	X [m]	X [m]	X	X		X	X
Monitor for fluid loss including from diarrhea & possible *C. difficile* [b]	X	X	X	X	X	X	X	X	X	X	X	X [q]	X	X

Notes:

a Tumor lysis labs to include: electrolytes (sodium, potassium, chloride, carbon dioxide); creatinine, calcium, lactate dehydrogenase (LDH), uric acid, K+ levels

b Alvocidib can induce diarrhea when given over a short period of time. Dehydration from diarrhea can exacerbate the morbidity associated with tumor lysis syndrome (ie, acute renal failure). Over-the-counter measures are typically effective in this setting if initiated early. It is strongly suggested that patients take 2 tablets of loperamide, 2 mg each (or equivalent), prior to the alvocidib IVI and then take 1 tablet (2 mg) for every loose stool up to a maximum of 8 tablets (16 mg) in a 24-hr period. If loperamide (or equivalent) does not control diarrhea, diphenoxylate hydrochloride with atropine sulfate (or equivalent) 5 mg orally four (4) times daily may be added. If diarrhea is not controlled with either prophylactic regimens and is ≥Grade 2, patients should contact the clinic and study drug treatment should be held until diarrhea has resolved.

c Mandatory oral allopurinol daily from Days 1-14 of Cycle 1.

d Administer pretreatment IV hydration prior to first AZA dose per institutional standards.

e Oral phosphate binder daily from Days 1-14 of Cycle 1 (see *Section 4.5.1.1.2*)

f Tumor lysis labs required just prior to D1 AZA dose and 2 hrs post D1 AZA dose

g Potassium levels obtained as part of the tumor lysis labs at 2 hrs post AZA dose should be reviewed immediately for indications of tumor lysis syndrome as additional treatment may be warranted (footnote 'h').

h If K+ 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 (1 ampule) of IV dextrose 50%, unless there are other likely causes of hyperkalemia other than TLS or contraindication to its use. Investigators are strongly encouraged to consider patient hospitalization for inpatient monitoring and follow up. 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.

i Tumor lysis labs to also be drawn on Days 2 and 3 prior to AZA doses during Cycle 1.

j Mandatory IV hydration with 0.45% NaCl (or similar hydration fluid per institutional standard) sterile solution at 500 cc for 1-2 hrs prior to alvocidib, then an additional 500 cc for 1-2 hrs after alvocidib during Cycle 1 (volume may be reduced to between 250 cc – 500 cc, if clinically indicated). Hydration is optional for subsequent cycles.
k Continue oral phosphate binder daily on Days 1-14 of Cycle 1.
l Assess tumor lysis labs prior to alvocidib IVI and 2 hrs (±30 mins) after completion of IV hydration post alvocidib.
m Potassium levels obtained as part of the tumor lysis lab panel at 2 hrs after completion of IV hydration post alvocidib should be reviewed immediately for indications of tumor lysis syndrome as additional treatment may be warranted (footnote 'h').
n Tumor lysis labs to be drawn daily for the first 2 days following alvocidib IVI (ie, Days 11-12) and at least weekly for the remainder of Cycle 1.
o Oral allopurinol may be discontinued for subsequent treatment cycles if uric acid levels are within normal limits and there is no evidence of TLS.
p Oral phosphate binder may be discontinued for subsequent treatment cycles if serum phosphorus levels are <3 after the first treatment with alvocidib and there is no evidence of TLS
q If diarrhea persists beyond Cycle 1, patients should undergo testing for *C. difficile*. Should testing indicate the presence of *C. difficile*, appropriate antibiotics targeting this infection should be initiated. Should testing exclude the presence of *C. difficile*, diarrhea prophylaxis similar to Cycle 1 should be continued in subsequent cycles.
r Assess tumor lysis labs just prior to C2D1 AZA dose and 2 hours post C2D1 AZA dose
s Assess tumor lysis labs just prior to C2D10 alvocidib IVI and 2 hrs (±30 mins) after completion of alvocidib IVI
t During Cycles 3+, TLS labs to be checked at the discretion of the investigator in relation to patient blast counts

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 – NEW YORK HEART ASSOCIATION CLASSIFICATION OF HEART FAILURE

NYHA Class	Definition	Limitation	Example
I	Ordinary physical activity does not cause undue fatigue, dyspnea, palpitation	None	Can complete any activity requiring ≤ 7 mets: • Carry 11 kg up 8 steps • Carry objects weighing 36 kg • Shovel snow • Spade soil • Ski • Play squash, handball or basketball • Jog/walk 8 km/h
II	Ordinary physical activity causes fatigue, dyspnea, palpitation, or angina	Slight	Can complete any activity requiring ≤ 5 mets: • Sexual intercourse without stopping • Garden • Roller skate • Walk 7 km/h on level ground • Climb one flight of stairs at a normal pace without symptoms
III	Comfortable at rest; less than ordinary physical activity causes fatigue, dyspnea, palpitation, or angina	Moderate	Can complete any activity requiring ≤ 2 mets: • Shower or dress without stopping • Strip and make bed • Clean windows • Play golf • Walk 4 km/h
IV	Symptoms at rest; any physical activity increases discomfort	Severe	Cannot do or cannot complete any activity requiring ≥ 2 mets; cannot do any of the above activities

Mets = metabolic equivalents
Reference: http://www.merck.com/mmpe/sec07/ch074/ch074a.html#CEGDEIFG

APPENDIX D – NCI COMMON TERMINOLOGY CRITERIA FOR ADVERSE EVENTS

View the National Cancer Institute Common Terminology Criteria for Adverse Events v5.0 (NCI CTCAE) criteria electronically at the following Web site:

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

APPENDIX E – LABORATORY TESTS

Hematology*	• CBC with manual differential •	• Platelet Count •
	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.	

Full Serum Chemistry Panel*	• 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 (CO_2)

Tumor Lysis Labs	• Electrolytes
	  ○ Sodium
	  ○ Potassium
	  ○ Chloride
	  ○ CO_2
		• Creatinine
		• Calcium
		• LDH
		• Uric acid
		• Phosphorus

Coagulation Panel	• Prothrombin time (PT)	• Activated partial thromboplastin time (aPTT)
Cardiac Tests	• 12-lead Electrocardiogram (ECG) with QTc	
Disease Status, Standard Cytogenetics & Response	• ***Bone marrow biopsy and/or aspiration and peripheral blood (including peripheral blasts)*** • ***Samples to be analyzed at local laboratories***	
Pharmacodynamic Analyses	• ***Bone marrow biopsy and/or aspiration and peripheral blood*** • BH3 profiling with an emphasis on MCL-1 dependence, genetic mutations, and other biomarkers associated with MDS • ***Samples to be analyzed at a central laboratory (see Reference Laboratory Manual for additional information)***	
Pharmacokinetic Analyses	• C_{max}, T_{max}, AUC_{0-24}, $t_{1/2}$, AUC_t, AUC_{0-inf}, and CL	
Other Tests	• Pregnancy test (urine or serum determination of β-hCG in females of childbearing potential)	

* During each cycle, it is critical to record all laboratory values for hematology and serum chemistry parameters collected during the time the patient is on study to facilitate change-from-baseline analyses. Any laboratory parameters assessed outside the per-protocol visits (ie, interim labs) will be recorded as 'Unscheduled Laboratory Assessments' in the database.

APPENDIX F – REVISED INTERNATIONAL WORKING GROUP (IWG) RESPONSE CRITERIA (2006)

Category	Response criteria (responses must last at least 4 wk)
Complete remission	Bone marrow: ≤ 5% myeloblasts with normal maturation of all cell lines*
	Persistent dysplasia will be noted*†
	Peripheral blood‡
	Hgb ≥ 11 g/dL
	Platelets ≥ 100 × 10^9/L
	Neutrophils ≥ 1.0 × 10^9/L†
	Blasts 0%
Partial remission	All CR criteria if abnormal before treatment except:
	Bone marrow blasts decreased by ≥ 50% over pretreatment but still > 5%
	Cellularity and morphology not relevant
Marrow CR†	Bone marrow: ≤ 5% myeloblasts and decrease by ≥ 50% over pretreatment†
	Peripheral blood: if HI responses, they will be noted in addition to marrow CR†
Stable disease	Failure to achieve at least PR, but no evidence of progression for > 8 wks
Failure	Death during treatment or disease progression characterized by worsening of cytopenias, increase in percentage of bone marrow blasts, or progression to a more advanced MDS FAB subtype than pretreatment
Relapse after CR or PR	At least 1 of the following:
	Return to pretreatment bone marrow blast percentage
	Decrement of ≥ 50% from maximum remission/response levels in granulocytes or platelets
	Reduction in Hgb concentration by ≥ 1.5 g/dL or transfusion dependence
Cytogenetic response	Complete
	Disappearance of the chromosomal abnormality without appearance of new ones
	Partial
	At least 50% reduction of the chromosomal abnormality

APPENDIX F – INTERNATIONAL WORKING GROUP (IWG) RESPONSE CRITERIA (2006) (CONT)

Category	Response criteria (responses must last at least 4 wk)
Disease progression	For patients with: Less than 5% blasts: ≥ 50% increase in blasts to > 5% blasts 5%-10% blasts: ≥ 50% increase to > 10% blasts 10%-20% blasts: ≥ 50% increase to > 20% blasts 20%-30% blasts: ≥ 50% increase to > 30% blasts Any of the following: At least 50% decrement from maximum remission/response in granulocytes or platelets Reduction in Hgb by ≥ 2 g/dL Transfusion dependence
Survival	Endpoints: Overall: death from any cause Event free: failure or death from any cause PFS: disease progression or death from MDS DFS: time to relapse Cause-specific death: death related to MDS

To convert hemoglobin from grams per deciliter to grams per liter, multiply grams per deciliter by 10.

MDS indicates myelodysplastic syndromes; Hgb, hemoglobin; CR, complete remission; HI, hematologic improvement; PR, partial remission; FAB, French-American-British; AML, acute myeloid leukemia; PFS, progression-free survival; DFS, disease-free survival.

*Dysplastic changes should consider the normal range of dysplastic changes (modification).[41]

†Modification to IWG response criteria.

‡In some circumstances, protocol therapy may require the initiation of further treatment (eg, consolidation, maintenance) before the 4-week period. Such patients can be included in the response category into which they fit at the time the therapy is started. Transient cytopenias during repeated chemotherapy courses should not be considered as interrupting durability of response, as long as they recover to the improved counts of the previous course.

APPENDIX F – INTERNATIONAL WORKING GROUP (IWG) RESPONSE CRITERIA (2006) (CONT)

Hematologic improvement*	Response criteria (responses must last at least 8 wk)†
Erythroid response (pretreatment, < 11 g/dL)	Hgb increase by ≥ 1.5 g/dL Relevant reduction of units of RBC transfusions by an absolute number of at least 4 RBC transfusions/8 wk compared with the pretreatment transfusion number in the previous 8 wk. Only RBC transfusions given for a Hgb of ≤ 9.0 g/dL pretreatment will count in the RBC transfusion response evaluation†
Platelet response (pretreatment, $< 100 \times 10^9$/L)	Absolute increase of $\geq 30 \times 10^9$/L for patients starting with $> 20 \times 10^9$/L platelets Increase from $< 20 \times 10^9$/L to $> 20 \times 10^9$/L and by at least 100%†
Neutrophil response (pretreatment, $< 1.0 \times 10^9$/L)	At least 100% increase and an absolute increase $> 0.5 \times 10^9$/L†
Progression or relapse after HI‡	At least 1 of the following: At least 50% decrement from maximum response levels in granulocytes or platelets Reduction in Hgb by ≥ 1.5 g/dL Transfusion dependence

Deletions to the IWG response criteria are not shown.
To convert hemoglobin levels from grams per deciliter to grams per liter, multiply grams per deciliter by 10.
Hgb indicates hemoglobin; RBC: red blood cell; HI: hematologic improvement.
*Pretreatment counts averages of at least 2 measurements (not influenced by transfusions) $\geq$ 1 week apart (modification).
†Modification to IWG response criteria.
‡In the absence of another explanation, such as acute infection, repeated courses of chemotherapy (modification), gastrointestinal bleeding, hemolysis, and so forth. It is recommended that the 2 kinds of erythroid and platelet responses be reported overall as well as by the individual response pattern.

[28]