CITY OF HOPE MEDICAL CENTER
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DEPARTMENT OF HEMATOLOGY AND HCT

TITLE: A Phase I/II Trial of 8-Chloro-adenosine in Relapsed or Refractory Acute Myeloid Leukemia

CITY OF HOPE PROTOCOL VERSION: IRB # 14269 PROTOCOL DATE: 07/27/2020

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SITE: AML

STAGE (If applicable): Relapsed/Refractory

MODALITY: Chemotherapy

TYPE: Phase I/II

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Clinical Trial Protocol

A Phase I/II Trial of 8-Chloro-adenosine in Relapsed or Refractory Acute Myeloid Leukemia

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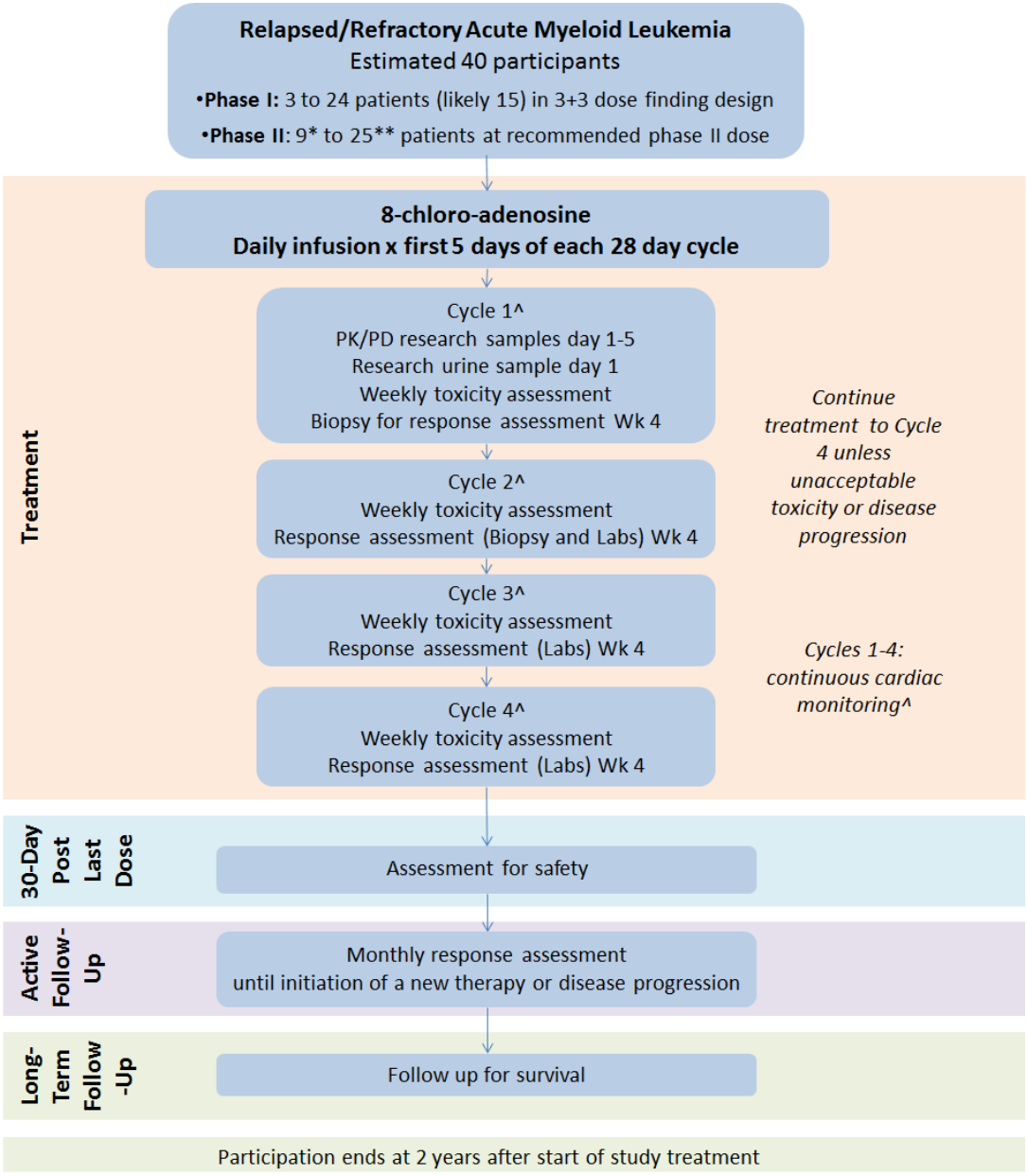
Agent(s): 8-chloro-adenosine
IND#: 118750

IND holder: City of Hope

EXPERIMENTAL DESIGN SCHEMA

This is a phase I/II trial of 8-chloro adenosine when given as a single agent in patients with relapsed or refractory acute myeloid leukemia.



*Six of the 9 initial participants included in the phase II will be phase I participants treated at the RP2D.

**In the phase II, if 0 responses are seen in the first 9 participants treated at the RP2D, the study will be terminated. If at least 1 participant responds, the phase to continue to enroll up to 25 participants treated at the RP2D.

^Participants will be hospitalized during therapy (at minimum the first 6 days of the cycle). Participants will be admitted to the telemetry unit and undergo continuous cardiac monitoring from start of treatment until 24 hours after completion of therapy during Cycles 1-4.

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PROTOCOL SYNOPSIS

Protocol Title:
A Phase I/II Trial of 8-chloro-adenosine in Relapsed or Refractory Acute Myeloid Leukemia
Brief Protocol Title for the Lay Public (if applicable):
8-chloro-adenosine in patients with relapsed or refractory acute myeloid leukemia
Study Phase:
Phase I/II
Participating Sites:
Single Center: City of Hope, Duarte CA
Rationale for this Study:
8-chloro-adenosine is a derivative and active metabolite of 8-chloroadenosine 3'5'-monophosphate (8-chloro-cAMP). The latter compound has shown antitumor activity in a broad variety of cancers in preclinical studies as well as previous clinical trials. 8-chloro-adenosine has also shown antineoplastic activity in vitro and in vivo with an excellent toxicity profile in preclinical studies. 8-chloro-adenosine appears to have a novel mechanism of action. Its cytotoxic metabolite, 8-chloro-ATP induces depletion of cellular ATP stores and interferes with RNA synthesis thereby leading to apoptosis. Among all cell lines tested in vitro, 8-chloro-adenosine has been shown to be particularly active in leukemia including human AML cell lines and AML primary cells. Efficacy has recently been demonstrated in an animal model of AML. Importantly, the pathway of 8-Cl-Ado-mediated cell death appears to be p53-independent, which is especially critical considering that p53 mutations/deletions are associated with drug resistance and adverse outcomes in AML. Furthermore, 8-chloro-adenosine synergizes with the chemotherapy drugs clofarabine, cytarabine and doxorubicin in the induction of growth inhibition of human AML cells, thereby potentially expanding the therapeutic potential of this novel agent. The in vitro antileukemic activity, favorable toxicity profile and unique mechanism of action makes 8-chloro-adenosine an ideal candidate for testing in early phase clinical trials in AML.
Objectives:
<u>Phase I</u> *Primary Objective* To determine the maximum tolerated dose (recommended phase II dose, RP2D) of 8-chloro-adenosine, when given as a single agent, in patients with relapsed or refractory acute myeloid leukemia. To assess tolerability and safety of 8-chloro-adenosine at each dose level by evaluation of toxicities including: type, frequency, severity, attribution, time course and duration. *Secondary Objective* To evaluate for disease response to 8-chloro-adenosine in refractory/relapsed AML on each dose level tested.

Phase II

Primary Objective

To estimate the response rate and to evaluate the antitumor activity of 8-chloro-adenosine, when given as a single agent, as assessed by complete remission rate (CR+CRi) at the RP2D.

Secondary Objectives

To obtain estimates of remission duration and survival probabilities (overall and event-free).

To obtain an estimate of the overall response rate (CR+CRi+PR).

To summarize and evaluate toxicities by type, frequency, severity, attribution, time course and duration.

Clinical Pharmacology Objectives (Phase I and II)

To describe the plasma, urinary and cellular pharmacokinetics of 8-chloro-adenosine and metabolites.

To determine the impact of 8-chloro-adenosine on cellular ATP pool in AML blasts.

To assess the impact of 8-chloro-adenosine therapy on select short-lived mRNAs and corresponding proteins in circulating AML blasts.

To correlate clinical responses and toxicity with plasma/urine 8-chloro-adenosine level (PK), cellular 8-Cl-ATP (PK) and cellular ATP pool.

Exploratory Ex-Vivo Molecular Objectives (Phase I and II)

To determine the cytotoxicity of 8-chloro-adenosine toward leukemic progenitor cells in vitro.

To generate a preliminary pre-treatment RNA/miRNA signature in leukemic progenitor cells, and explore its possible association with in vitro cytotoxicity to 8-chloro-adenosine.

To explore the possible association between the preliminary RNA/miRNA signature and clinical response to 8-chloro-adenosine.

Study Design:

This study will be conducted as a single center phase I/II trial.

Participants will receive up to four cycles of study agent, and will be followed for up to 2 years after their first dose of study agent.

Phase I Design:

The phase I portion will follow a modified 3+3 design in order to evaluate toxicities associated with 8-chloro-adenosine when given as a single agent. In the absence of notable toxicity (see sections 13.2 and 13.3), four doses of 8-chloro-adenosine will be tested: 100mg/m^2; 200 mg/m^2; 400 mg/m^2 and 800 mg/m^2. Intermediate dose levels ('b' levels), in increments of 50% from the planned four doses, will be explored in the presence of dose limiting toxicity or unacceptable moderate toxicity. Intermediate doses are defined as follows: 150mg/m^2; 300 mg/m^2; and 600 mg/m^2.

With amendment v07, the starting dose will be: 400 mg/m^2 (dose level 3a.

8-chloro-adenosine will be administered as a 4 hour infusion daily for 5 days every 4 weeks; treatment cycle length is 28 days. The dose of 8-chloro-adenosine will depend on dose level assignment. The maximum tolerated dose (MTD) will be established by evaluating dose limiting

toxicity (DLT) during cycle 1. The highest dose level that produces ≤1/6 DLTs in cycle 1 will be the MTD.

The recommended phase II dose (RP2D) will generally be the MTD, but it may be less than the MTD based on a review of available data/cumulative toxicities and activity.

Phase II Design:

The phase II portion of this study will implement a Gehan two-stage design to estimate the response rate and to evaluate the activity of 8-chloro-adenosine when given as a single agent (Gehan, 1961). The phase II portion of the study is expected to enroll a minimum of 9 and a maximum of 25 patients. Patients treated at the RP2D in the phase I portion of the study, who receive at least 2 cycles of treatment, will count toward the 25 patients required. The sample size is based on the desire to estimate the response rate with at most 10% standard error, and early stopping if the treatment is unexpectedly ineffective.

At stage 1, 9 patients will be entered on the study. If 0 responses are seen in the first 9 patients treated, the study will be terminated and the true regimen response will be declared ≤30%. If at least 1 patient responds, the trial will continue to the second stage. Because patients treated during the phase I portion of the trial at the dose selected for the phase II trial will be counted (n=6), only 3 additional patients will be enrolled at stage 1. Under this design if the study agent is >30% effective, there would be 96% chance of at least one success.

At stage 2, 16 additional patients will be entered. This accrual provides for estimation of the response rate with no more than 10% standard error.

Endpoints:

Phase I:

The primary endpoint is toxicity. Toxicity will be graded according to the NCI-Common Terminology Criteria for Adverse Events version 4.03. Dose limiting toxicity (DLT) and moderate toxicity are defined in section 13.2 of the protocol. Note: The phase II portion of the study will use the DLT definition to define unacceptable toxicity. The MTD will be based on the assessment of DLT during cycle 1. All patients who are not evaluable for dose limiting toxicity will be replaced.

Phase II:

The primary endpoint is complete remission rate (CR/CRi) and is based on the Döhner et al. 2010 criteria[24]. Response will be assessed at the end of each cycle/just prior to the start of each cycle. Secondary endpoints include: remission duration, survival (overall and event-free) and toxicity.

Clinical Pharmacology Endpoints:

- For plasma PK, levels of 8-chloro-adenosine, its deaminated metabolite, 8-chloro-inosine, and its base 8-chloro-adenine will be quantitated in plasma during 5 days of therapy.
- For cellular PK, concentrations of 8-chloro-adenosine and 8-chloro-ATP will be quantitated in circulating leukemia cells in the peripheral blood.
- For urine PK, levels of 8-chloro-adenosine, its deaminated metabolite, 8-chloro-Inosine, and its base 8-chloro-adenine will be quantitated in urine during the first 24 hours of therapy.
- For PD, the impact of therapy on endogenous ATP pools, as well as the effect on short-lived mRNA transcripts and proteins will be analyzed in circulating acute leukemia blasts.

Sample Size:

The overall expected study sample size is 43.

While the phase I study is expected to enroll and treat 15 patients (dose levels 1-3(a) to enroll/treat 3 patients each for a total of 9, and another 6 at dose level 4a-assuming the 800mgm^2 dose is well tolerated), a maximum of 24 patients could be treated (6 patients treated at each of the four dose levels –'a' levels) assuming intermediate doses are not pursued.

The phase II portion of the study is expected to enroll a minimum of 9 (6 of whom will be from the phase I) and a maximum of 25 patients.

Estimated Duration of the Study

Accrual, for both phases, is expected to be completed in 70 months. Follow up of each patient will be for a maximum of 2 years; the maximum study duration is 8 years.

Summary of Subject Eligibility Criteria:

Inclusion criteria

- Diagnosis of AML as per WHO Classification of Hematologic Neoplasms.
- Meets one of the three criteria:
    - Relapsed AML who have failed at least 1 line of salvage therapy
    - De novo AML who have not achieved CR after 2 lines of therapy
    - AML evolving from MDS or myeloproliferative disorder who have failed hypomethylating agent and/or induction chemotherapy
    - Patients who have relapsed after allogeneic HCT are eligible if they are at least 3 months after HCT, do not have active GVHD and are off immunosuppression except for maintenance dose of steroids (prednisone 10 mg/day or less).
- At least 2 weeks from prior chemotherapy or radiation therapy to time of start of treatment, except for hydroxyurea or corticosteroid therapywhich may be continued through Cycle 1 of Phase 1 portion of protocol.
- Age ≥ 18 and life expectancy > 3 months.
- ECOG status of 0-2.
- AST and ALT ≤ 2.5 x ULN
- QTc ≤ 480 ms
- Calculated creatinine clearance (CrCl) ≥ 50 mL/min per 24 hour urine

Exclusion criteria

- Expected to undergo HCT within 120 days of enrollment.
- Current or planned use of agents that prolong or suspected to prolong QTc
- Diagnosis of acute promeylocytic leukemia
- Active central nervous system leukemia
- Active fungal infection
- History of heart failure or cardiac arrhythmia
- Females who are pregnant or lactating.

Investigational Product Dosage and Administration:
Four doses of 8-chloro-adenosine may be tested (dose level 1a: 100mg/m^2; dose level 2a: 200 mg/m^2; dose level 3a: 400 mg/m^2 and dose level 4a: 800 mg/m^2 Intermediate dose levels –'b' levels, in increments of 50% from the planned four doses, will be explored in the presence of dose limiting toxicity or unacceptable moderate toxicity. Intermediate doses are defined as follows: 150mg/m^2; 300 mg/m^2; and 600 mg/m^2. **With amendment v07**, the starting dose will be 400 mg/m^2 (dose level 3a). 8-chloro-adenosine will be administered as a 4-hour intravenous infusion for 5 days every 28 days). There will be no intra patient dose escalation.
Clinical Observations and Tests to be Performed:
Clinical observations will include tolerance as well as response to treatment (see study calendar). Baseline studies will include a history and physical,12-lead EKG, bone marrow biopsy as well as a laboratory evaluation to include CBC with differential, comprehensive chemistry panel (to include LDH, magnesium, phosphorus and uric acid) and urinalysis. Response will be assessed at the end of every cycle. Subjects will be evaluated through weekly clinic visits and physical exams as well as laboratory tests to include CBC with differential, comprehensive chemistry panel and labs. 12-lead EKG will be performed at pre-infusion and post-end of infusion on days the study drug is administered for all cycles. Patients will undergo continuous cardiac monitoring in telemetry unit from start of treatment until 24 hours after completion of treatment during Cycles 1-4. Blood and urine samples will be collected for pharmacokinetic and pharmacodynamic correlative studies. In addition, additional bone marrow will be collected at the time of bone marrow biopsy for planned correlative studies and future studies.
Statistical Considerations:
<u>Study Design</u> *Phase I:* The primary objective of the phase I study is to determine the recommended phase II dose (RP2D) of 8-chloro-adenosine when given as a single agent, in patients with relapsed or refractory acute myeloid or lymphoblastic leukemia. The phase I study will follow a modified 3+3 design for enrollment with dose escalation, or expansion of a cohort on the basis of the occurrence of dose limiting toxicities (DLTs) during cycle 1. This modified design involves an initial accelerated dose escalation stage (dose-doubling from an initial dose of 100mg/m^2 to 800mg/m^2) that may be ended by a single DLT in cycle 1 or two occurrences of 'moderate' treatment-related toxicity. During the initial accelerated stage of accrual, cohorts of up to 3 patients will be enrolled and the dose level will be escalated by increments of 100%. The accelerated stage will end when two patients encounter moderate toxicity or when one patient encounters DLT during cycle 1. At the conclusion of the accelerated stage, the dose escalation will occur according to the standard 3+3 rules: The highest dose level that produces ≤1/6 DLTs in cycle 1 will be the maximum tolerated dose (MTD). The RP2D of 8-chloro-adenosine will generally be the MTD, but it may be less than the MTD based on a review of available data/cumulative toxicities from phase I. The RP2D identified in the phase I portion of the study will be brought forward for further activity evaluation in the phase II portion.

Phase I/ Evaluable for Toxicity: Patients will be considered evaluable for toxicity if they receive any study drug. To be evaluable in the context of dose escalation, a patient must receive at least 80% of 8-chloro-adenosine and be followed for at least 28 days during cycle 1 or experience a DLT during the first cycle of therapy. All patients who are not evaluable for dose limiting toxicity will be replaced. Patients who are replaced with a moderate toxicity will be used in the decision to stop the accelerated dose-doubling stage.

Phase II: The primary objective is to estimate the response rate and to evaluate the antitumor activity of 8-chloro-adenosine, in patients with relapsed or refractory acute myeloid or lymphoblastic leukemia. The primary endpoint is complete remission rate (CR/CRi) and is based on the Döhner, 2010 criteria[24]. A cycle of treatment is 28 days. Each patient's disease status will be evaluated at baseline. Response will be assessed at the end of each cycle/just prior to the start of each cycle.

The phase II portion of this study will implement a Gehan two-stage design to estimate the response rate and to evaluate the activity of 8-chloro-adenosine when given as a single agent. The phase II portion of the study is expected to enroll a minimum of 9 and a maximum of 25 patients. The six patients treated at the RP2D in the phase I portion of the study will count toward the 25 patients required; given this, we expect to enroll only 19 new patients on the phase II trial. The sample size is based on the desire to estimate the response rate with at most 10% standard error, and early stopping if the treatment is unexpectedly ineffective.

At stage 1, 9 patients will be entered on the study. If 0 responses are seen in the first 9 patients treated, the study will be terminated and the true regimen response will be declared ≤30%. If at least 1 patient responds, the trial will continue to the second stage. Because patients treated during the phase I portion of the trial at the dose selected for the phase II trial will be counted (n=6), only 3 additional patients will be enrolled at stage 1. Under this design if the study agent is >30% effective, there would be 96% chance of at least one success.

At stage 2, 16 additional patients will be entered. This accrual provides for estimation of the response rate with no more than 10% standard error.

Phase II (Evaluable for Toxicity): Patients will be considered evaluable for toxicity if they receive any study drug. Patients enrolled/treated as part of the phase II study will not be replaced based on toxicity.

Phase I and II (Evaluable for Response): For the phase II portion, as part of the primary analysis, patients will be considered evaluable for response if they are eligible, have baseline disease assessments, and receive 2 cycles of protocol treatment or achieve a CR/CRi after 1 cycle of protocol treatment. Patients will have their response classified according to the Dohner 2010 criteria[24].

<u>Sample Size</u>

Phase I: In the phase I portion of this study, the total sample size will depend on the number of dose levels evaluated to determine the RP2D. While the phase I study is expected to enroll and treat 15 patients (dose levels 1-3 –'a' levels, to enroll/treat 3 patients each for a total of 9, and another 6 at dose level 4a-assuming the 800 mg/m^2 dose is well tolerated), a maximum of 24 patients could be treated (6 patients treated at each of the four dose levels) assuming intermediate doses are not pursued.

Phase II: The phase II portion of the study is expected to enroll a minimum of 9 and a maximum of 25 patients. The six patients treated at the RP2D in the phase I portion of the study will count toward the 25 patients required; given this, we expect to enroll only 19 new patients during the

phase II trial portion. The sample size is based on the desire to ensure a (promising) response rate of 30% is not missed.

Clinical Pharmacology (PK/PD): Pharmacokinetic (PK) analyses will be performed during Cycle 1 in all enrolled patients in Phase 1 and Phase 2 portions of this protocol. In addition, pharmacodynamic measurements will be performed during Cycle 1 in all enrolled patients in the Phase 1 and 2 portions who have ≥5000 circulating leukemic blasts per microliter.

Molecular Studies: For mRNA-seq, sequence reads will be mapped to the human genome (hg19) using TopHat and the frequency of Refseq genes will be counted with customized R scripts. For miRNA-seq, sequences will be adapter trimmed and aligned to hg19 genome using Novoalign, and miRNA expression level will be counted as previously described [1]. The raw counts will then be normalized and compared using the Bioconductor package "edgeR" [2]. Fold changes > than 3 with a false discovery rate (FDR) < 0.05 will be considered significant. Assuming the dispersion of genes across replicates is 0.2, 12 samples in each group (responder vs. non-responder) should have 88% power to detect a 3-fold difference between groups at an FDR level of 0.05.

Safety Analysis and Stopping Rules for Excessive Toxicity

The early stopping rule for safety/toxicity will be assessed for each patient after cycle 1: the expected rate of unacceptable toxicity should not be ≥33%. Note: The phase II portion of the study will use the DLT definition to define unacceptable toxicity. If more than the specified number of patients has significant treatment related toxicities, patient accrual will be halted and a full review of the data by the Data Safety Monitoring Committee (DSMC) will be mandated. Patient accrual will not resume until approved by the DSMC to do so.

In addition, cumulative safety data will be reviewed on a quarterly basis in order to identify safety concerns that may emerge due to cumulative exposure.

Trial Statistical Analysis Plan

Patient demographic and baseline characteristics, including age, gender, medical history, and prior therapy, will be summarized using descriptive statistics. For continuous variables, descriptive statistics (number [n], mean, standard deviation, standard error, median (range) will be provided. For categorical variables, patient counts and percentages will be provided.

Phase I Analysis: Observed toxicities will be summarized in terms of type (organ affected or laboratory determination), severity, time of onset, duration, probable association with the study treatment and reversibility or outcome. Baseline information (e.g. the extent/type of prior therapy) and demographic information will be presented as well to describe the patients treated in this study.

Phase II Analysis: The complete remission rate (CR+CRi) will be calculated as the percent of evaluable patients; exact 95% confidence intervals will be calculated for these estimates. Response rates will also be evaluated based on number and type of prior therapy(ies). Time to response, duration of response, and survival will be estimated using the product-limit method of Kaplan and Meier.

Clinical Pharmacology Analysis: Plasma, urinary and cellular PK of 8-chloro-adenosine and metabolites will be quantitated as continual measurement variables. Descriptive statistics and graphical displays will be used to summarize levels of serum/urine 8-chloro-adenosine and its metabolites at each time point and to evaluate changes over time. A paired t-test will be used to determine if there is a statistically significant change. Similar methods will be used to assess the PD impact of 8-chloro-adenosine on the cellular ATP pool and select short-lived mRNAs including corresponding proteins in AML blasts. Protein and mRNA levels will be summarized descriptively

using means, medians, standard deviations and ranges. Summary statistics of the PK/PD parameters for the population will be derived from the parameters obtained from the individual patients. Results will also be summarized in terms of response and toxicity. To determine if there are dose-dependent changes in 8-Cl-ATP and 8-chloro-adenosine (when administered ex vivo), summary statistics and graphical displays will be generated to descriptively compare accumulation of 8-Cl-ATP and endogenous ATP levels in circulating AML blasts over 5 doses. *Molecular Studies Analysis:* Gene ontology (GO) and pathways will be analyzed using DAVID (Database for Annotation, Visualization, and Integrated Discovery), an online bioinformatic resource for functional interpretation of large lists of genes. Pathways and GO that correlate with response will be sorted by their multiple comparison adjusted p value. Potential miRNA targets will be identified using TargetScan and their functional annotation will be done using DAVID. All summaries will be exploratory in nature, with the goal of developing further questions regarding the modulation of therapy, or regarding reasons for efficacy or lack of efficacy.
Sponsor/Licensee:
Investigator Initiated: City of Hope
Case Report Forms
Medidata RAVE® Electronic Data Capture system.

TABLE OF CONTENTS

ABBREVIATIONS

Abbreviation	Meaning
AE	Adverse Event
AML	Acute Myeloid Leukemia
CFR	Code of Federal Regulations
CICSL	Clinical Immunobiology Correlative Studies Laboratory
CLL	Chronic Lymphocytic Anemia
COH	City of Hope
CR	Complete Remission
CRA/CRC	Clinical Research Associate/Coordinator
CRF	Case Report Form
CRi	Complete Remission with Incomplete Blood Count Recovery
CTCAE	Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
DLT	Dose Limiting Toxicity
DHODH	Dihydroorotate Dehydrogenase
DSMC	Data Safety Monitoring Committee
EOI	End of Infusion
EOT	End of Treatment
FDA	Food and Drug Administration
GCP	Good Clinical Practice
IB	Investigator Brochure
ICD	Informed Consent Document
IDS	Investigational Drug Services
IND	Investigational New Drug
IRB	Institutional Review Board
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
PCA	Perchloric Acid
PD	Progressive Disease
PI	Principal Investigator
PMT	Protocol Monitoring Team
PR	Partial Response
RT	Room Temperature
SAE	Serious Adverse Event
SD	Stable Disease

1.0 GOALS AND OBJECTIVES (SCIENTIFIC AIMS)

1.1 Primary Objectives

Phase I:

To determine the maximum tolerated dose (recommended phase II dose, RP2D) of 8-chloro-adenosine, when given as a single agent, in patients with relapsed or refractory acute myeloid leukemia.

To assess tolerability and safety of 8-chloro-adenosine at each dose level by evaluation of toxicities including: type, frequency, severity, attribution, time course and duration.

Phase II:

To estimate the response rate and to evaluate the antitumor activity of 8-chloro-adenosine, when given as a single agent, as assessed by complete remission rate (CR+CRi).

1.2 Secondary Objectives

Phase I:

To evaluate for disease response to 8-chloro-adenosine in refractory/relapsed AML on each dose level tested.

Phase II:

To obtain estimates of remission duration and survival probabilities (overall and event-free).

To obtain an estimate of the overall response rate (CR+CRi+PR).

To summarize and evaluate toxicities by type, frequency, severity, attribution, time course and duration.

1.3 Clinical Pharmacology Objectives (Phase I and II)

To describe the plasma, urinary and cellular pharmacokinetics of 8-chloro-adenosine and metabolites.

To determine the impact of 8-chloro-adenosine on cellular ATP pool in AML blasts.

To assess the impact of 8-chloro-adenosine therapy on select short-lived mRNAs and corresponding proteins in circulating AML blasts.

To correlate clinical responses and toxicity with plasma/urine 8-chloro-adenosine level (PK), cellular 8-chloro-ATP (PK) and cellular ATP pool.

1.4 Exploratory Ex-Vivo Molecular Objectives (Phase I and II)

To determine the cytotoxicity of 8-chloro-adenosine toward leukemic progenitor cells in vitro.

To generate a preliminary pre-treatment RNA/miRNA signature in leukemic progenitor cells, and explore its possible association with in vitro cytotoxicity to 8-chloro-adenosine.

To explore the possible association between the preliminary RNA/miRNA signature and clinical response to 8-chloro-adenosine.

2.0 BACKGROUND

2.1 Acute Myeloid Leukemia

Relapsed/refractory AML carries a poor prognosis, with majority of adult patients eventually succumbing to their disease [3, 4]. This is also true of AML arising from MDS which carries a particularly dismal prognosis. Although intensive therapies like HSCT can cure a subset of these patients, given their advanced age and comorbidities, many adults with these disorders are not often candidates for HSCT. Despite the poor overall outcomes of chemotherapy in relapsed/refractory AML, only a handful of new drugs have been approved for chemotherapy of AML in the last 2 decades. This is despite the fact that a large number of molecularly targeted therapies have been extensively tested in clinical trials [5]. Hence there is urgent need to develop additional novel agents for treatment of AML.

2.2 8-Chloro-adenosine

8-chloro-adenosine (8-Cl-Ado) is the active form of 8-chloro-adenosine 3'5'-monophosphate (8-chloro-cAMP). The latter compound has shown antitumor activity in a broad variety of cancers in preclinical studies as well as previous clinical trials [6-9]. Subsequently, it was shown that the antineoplastic activity of 8-chloro-cAMP was mediated by conversion to its metabolite 8-Cl-Ado [10-12]. Hence 8-Cl-Ado was used in subsequent studies. 8-chloro-adenosine has shown antineoplastic activity in vitro and in xenograft models with an excellent toxicity profile in preclinical studies [13-15]. 8-chloro-adenosine appears to have a novel mechanism of action. Its cytotoxic metabolite, 8-chloro-ATP induces depletion of cellular ATP stores and interferes with RNA synthesis thereby leading to apoptosis [16, 17]. Among all cell lines tested in vitro, 8-chloro-adenosine has been shown to be particularly active in acute leukemia cell lines including human AML cell lines and AML primary cells [18-20]. The in vitro antileukemic activity, favorable toxicity profile in animal studies as well as its unique mechanism of action makes 8-chloro-adenosine an ideal candidate for testing in early phase clinical trials in AML.

2.3 Overview and Rationale of Study Design

This will be a Phase I/II study of 8-chloro-adenosine administered as a single agent in patients with relapsed/refractory AML. The Phase I study will be conducted initially to determine the MTD following which the Phase II (expansion phase) will be conducted at the selected dose to test activity of 8-chloro-adenosine in acute leukemia. Toxicity profile of 8-chloro-adenosine will be determined. Pharmacokinetics and pharmacodynamics of 8-chloro-adenosine will also be studied.

The study design and starting dose level selected for this study are based on previous experience with this agent in a Phase I trial for chronic lymphocytic leukemia (CLL) conducted at MD Anderson Cancer Center. In that trial a dose of 101mg/m2 (administered as a 5-day infusion over 1 hour) was reached without DLT. The availability of highly effective targeted agents for CLL resulted in slower than anticipated accrual to the study; the trial was closed to further accrual before the maximum tolerated dose was determined (Dr.Varsha Gandhi, Personal Communication).

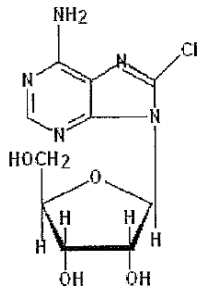
The first cohort of the Phase 1 portion of this trial will therefore start at 100mg/m2 infused over 1 hour daily for 5 days. Given the excellent toxicity profile observed so far doses will be doubled (by increments of 100% up to 800mg/m^2) in subsequent cohorts until MTD in order to quickly reach the biologically active dose as well as obtain some preliminary evidence of efficacy from the Phase I portion. Note: Intermediate dose levels, in increments of 50% from the planned four doses, will be explored in the presence of dose limiting toxicity or unacceptable moderate toxicity. Intermediate doses –'b' levels are defined as follows: 150mg/m^2; 300 mg/m^2; and 600 mg/m^2. **With amendment v07**, the starting dose will be 400 mg/m^2 (dose level 3a).

Extensive pharmacokinetic and pharmacodynamic studies are also incorporated into this protocol and may be helpful in determining the RP2D. A subsequent Phase II expansion phase will be conducted to determine the response rate in refractory/relapsed AML.

This study will be conducted in compliance with the protocol, Good Clinical Practice (GCP) and the applicable regulatory requirements.

2.4 Pharmacology of 8-Chloro-adenosine

2.4.1 Chemistry



Chemical Name: 8-chloro-adenosine

Other name: NSC 354258

Manufacturer: University of Iowa Pharmaceuticals

8-chloro-adenosine is an adenosine analogue with a ribose sugar and a chlorine group at the 8-position of the adenine base. 8-chloro-adenosine has a molecular formula C[10]H[12]N[5]O[4]Cl and molecular weight of 301.68. The melting point is 198-199°C. It is a white powder that has an intrinsic solubility in water of 4.8 mg/ml. It is stable (no degradation) at 25°C or 35°C for at least 2 months, however, it is unstable in water at pH<3 and pH>8. A 5 mg/ml aqueous solution appears to be stable at pH 7.0 for over 1 year.

8-chloro-adenosine was prepared in a 3-step reaction from adenosine: 1) acetylation of adenosine with acetic anhydride; 2) reaction with m-chloro-pherbenzoic acid/HCl to yield 8-chloro-adenosine triacetate; 3) deacetylation with sodium methoxide/methanol.

The drug is supplied at a concentration of 5 mg/mL in 5% dextrose (20 mL per vial).

2.4.2 Mechanism of Action

Nucleoside analogues have been developed into successful chemotherapeutic agents for a variety of hematologic and non-hematologic malignancies. One mechanism for generating nucleoside analogues is by adding a halogen group to the nucleoside. Halogenated nucleosides such as fludarabine, cladribine, and gemcitabine have proven clinical activity and multiple and differing mechanisms of action. Generally, these agents are taken up by cells, activated by phosphorylation, and incorporated into DNA by DNA polymerases. As such, they inactivate nucleic acid metabolism, repair, and processing pathways. Therefore, these halogenated nucleoside analogues are all directed at altering DNA metabolism.

8-chloro-adenosine is a halogenated ribonucleoside with promise for clinical application. Early studies demonstrated that 8-chloro-cyclic-adenosine-3',5'-monophosphate (8-chloro-cAMP) induced growth inhibition and apoptosis in several different malignant cell lines and primary tumor cell types [6-9]. Subsequently, it was demonstrated that one mechanism of action for 8-chloro-cAMP was through conversion to the active metabolite 8-chloro-adenosine [10-13]. 8-chloro-adenosine has anti-tumor

activity in various cell lines and r animal models including breast cancer, myeloma, colon cancer, neuroblastoma, glioma, and T cell acute lymphoblastic leukemia [13-22]; and our preliminary data demonstrate efficacy in an acute leukemia mouse model (See Section and Figure 5.2.1).

8-chloro-adenosine is taken up by cells and metabolized to mono, di-, and triphosphate. Accumulation of the cytotoxic metabolite of 8-chloro-adenosine, namely 8-chloro-ATP, results in depletion of cellular ATP stores, inhibition of RNA and protein synthesis, and induction of apoptosis. Incorporation of 8-chloro-adenosine into RNA may also contribute to the growth-inhibitory effect of this compound [16, 17]. Adenosine deaminase (ADA) converts 8-chloro-adenosine to 8-chloro-inosine and abrogates the cytotoxic effects of 8-chloro-cAMP, 8-chloro-AMP, and 8-chloro-adenosine [13].

The major mechanism of action for 8-chloro-adenosine does not appear to be through PKA1 inhibition as previously thought. Detailed mechanistic studies demonstrated that this analogue is incorporated into RNA at the 3'-terminus thereby aborting further synthesis. Incorporation was in all RNA species, with preferential incorporation into mRNA thereby interfering in RNA transcription [16, 17]. Studies in a colorectal cancer cell line, LS174T, indicate that 8-chloro-adenosine inhibits tumor cell growth by inducing G1 cell cycle arrest that is associated with large increases in $p21^{WAF1/Cip1}$ and p53 protein levels and a decrease in the phosphorylation status of the retinoblastoma protein [21]. Other studies with the colorectal cancer cell line HCT-116 demonstrated that 8-chloro-adenosine induces apoptosis that is independent of $p21^{WAF1/Cip1}$ and p53 proteins [14]. Recently, it was also shown that ATP depletion by 8-chloro-adenosine induces AMPK which in turn suppresses mTOR thereby leading to autophagic cell death [15].

In vitro studies with the CEM lymphoblastic leukemia model demonstrated that 8-chloro-adenosine induced growth inhibition and induction of apoptosis [19]. In this system, 8-chloro-adenosine induced significant increases in the level of cytosolic IKBα, thereby preventing NF-KB nuclear translocation. 8-chloro-adenosine also prevented TNF-α induced IKB decay and NF-KB activation in CEM cells [19]. Induction of DNA double stranded breaks by 8-chloro ATP mediated by topoisomerase II inhibition has been observed in the human myelocytic leukemia line K562 exposed to 8-chloro-adenosine [18].

Based on the above mentioned data, a RAID application for preclinical development of 8-chloro-adenosine was submitted by collaborating investigator Dr. Steven Rosen. The application was directed toward the development of 8-chloro-adenosine as a therapeutic agent for multiple myeloma. 8-chloro-adenosine was evaluated in vitro in the NIH 60 cell line screen and greater activity was seen in a leukemia sub-panel. The mean concentration inducing 50% growth inhibition (GI_{50}) for all cell lines in four separate assays ranged from 0.813 to 2.04 μM. In the standard two-day screen, all cell lines exhibited 50% growth inhibition (mean GI_{50}=1.74 μM). However, only 9 cell lines including 3/6 leukemia cell lines were totally growth inhibited at <100 μM. In a six-day assay, the mean GI_{50} concentration was 0.813 μM and 21 cell lines including 4/6 leukemia cell lines exhibited total growth inhibition (TGI) and 11 cell lines including 2/6 leukemia cell lines exhibited an LC_{50} of <100 μM. In vitro studies utilizing RPMI-8226 myeloma cells and SR immunoblastic leukemia cells demonstrated concentration and exposure time dependent killing with 8-chloro-adenosine. 8-chloro-adenosine was growth inhibitory and lethal in both cell lines with brief exposure (0.75 hr) at high concentrations: for RPMI-8226, TGI was 36 μM, LC50 was 51 μM; and for SR the TGI was 22 μM and LC50 was 29 μM. Lethality was achieved by longer exposures at lower concentrations: 9.96 μM for 24 hours was required for LC50 in RPMI-8226 and 8.4 μM for 12 hours was required for LC50 in SR cells.

8-chloro-adenosine showed cytotoxicity in 5 AML cell lines tested with median IC50 values ranging from 208 to 1372nM. 8-chloro-adenosine also inhibited proliferation of primary AML cells from de novo, relapsed and post-MDS AML. Drug sensitivity score (DSS) of 8-chloro-adenosine in AML samples was

higher compared to normal control bone marrow. Primary AML samples carrying *FLT3/ITD* mutation exhibited higher DSS compared to other AML subtypes.

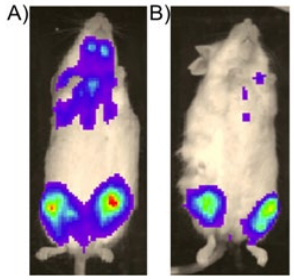
8-chloro-adenosine directly inhibits ribonucleotide reductase activity in an *in vitro* enzyme assay.

2.5 Preclinical Studies

2.5.1 Animal Efficacy Data

8-chloro-adenosine showed safety and efficacy in animal models of colorectal cancer, breast cancer, and AML. Athymic nude mice bearing HCT116 colorectal cancer xenografts experienced a 50% inhibition of tumor growth when treated with intraperitoneally injected 8-chloro-adenosine (50 mg/kg; twice per week for 4 weeks) as compared to untreated mice [14]. 8-chloro-adenosine inhibited the growth of two orthotopic mouse models of human breast cancer (MCF-7 or BT-474) [15]. In a mouse model of acute leukemia, tumor growth and metastasis was significantly inhibited in mice treated with 50 mg/kg/day 8-chloro-adenosine (Figure 2.5.1). In this model, Molm-14 AML cells stably expressing luciferase protein were i.v. injected into NSG mice (6 per group) 3 days before start of treatment. Sixteen days after start of treatment, average tumor size of the treatment group was about 3.5 times less than the control group (1,200,000 photons $\pm$ 600,000 vs. 4,200,000 photons $\pm$ 800,000). No signs of toxicity (weight loss) were seen in any of these mouse models.

Figure 2.5.1 8-Chloro-adenosine Inhibits Tumor Growth in a Mouse Model of AML



Mice were injected with Molm-14 AML cells 3 days before start of treatment. Images of mice treated with (A) vehicle control and (B)

2.5.2 Animal Pharmacology and Toxicology

Role of adenosine deaminase on pharmacokinetics

An *in vivo* pharmacology study was performed in CD2F1 mice to determine if the addition of deoxycoformycin (DCF) affected the plasma or cellular pharmacology of 8-chloro-adenosine [22]. DCF is an inhibitor of adenosine deaminase and potentially could prevent degradation of 8-chloro-adenosine to 8-chloro-inosine, the inactive metabolite. Levels of phosphorylated metabolites were assessed in blood mononuclear cells from mice that were not pretreated or pretreated with 2 mg/kg DCF 30 minutes prior to administration of 100 mg/kg of 8-chloro-adenosine. The cellular levels of 8-chloro-AMP, 8-chloro-ADP, and 8-chloro-ATP at one hour were 27-38% lower when mice were pretreated with DCF. Similar pharmacokinetic studies were performed in rats by administration of 8-chloro-adenosine 42.5 mg/kg IV with or without pretreatment with 4 mg/kg. Cellular pharmacology studies demonstrated lack of effect of DCF in augmenting formation of phosphorylated metabolites of 8-chloro-adenosine in blood mononuclear cells [2].

IND-directed GLP animal toxicology studies of 8-chloro-adenosine

Four IND-directed GLP animal toxicology studies were performed at M. D. Anderson Cancer Center in order to identify the toxicity spectrum of 8-chloro-adenosine, likely end organ toxicity, and a safe starting dose and administration schedule for the clinical trial. These studies were conducted in compliance with

guidelines for GLP for non-clinical laboratory studies as specified in the code of federal regulations (21CFR Part 58). These studies were conducted at the M. D. Anderson Cancer Center in the Pharmaceutical Development Center under the direction of Mary Johansen, PharmD. These studies include Study 8-CIA-01, Study 8-CIA-03, 8-CIA-02, and 8-CIA-04 are summarized as follows:

Study 8-ClA-01:

This was a dose range-finding study of 8-chloro-adenosine in mice. In this study, 10 male mice (5 per group) were assigned to a control group that received no study drug and a treatment group in which animals received 100 mg/kg IV bolus via tail vein daily for 5 consecutive days. Animals were sacrificed the day after the last dose to evaluate acute pathology and organ toxicity. No toxicities were observed in the treatment or control group. Subsequently, an additional 5 female mice received 8-chloro-adenosine 300mg/kg IV bolus daily for 5 consecutive days. Animals were sacrificed the day after the last infusion. In this group mild to moderate renal nephrosis was observed in 5/5 animals at this dose level. In this study, a no-observable-adverse effect level of 100mg/kg/day IV bolus daily for 5 consecutive days was identified. Based on these findings, Study 8-CIA-03 was designed and is a multiple dose toxicity study of 8-chloro-adenosine in mice.

Study 8-CIA-02:

This was a dose range-finding study of 8-chloro-adenosine in a single female dog to determine drug tolerability in this species. 8-chloro-adenosine was administered on 5 consecutive days with increasing dose given each day, as follows: 25, 50, 75, 100, 150mg/kg in short infusions. With this schedule and doses, gastrointestinal toxicity, characterized by vomiting, was observed at 100 and 150mg/kg. This resolved within 24 hours. Also, transient thrombocytopenia was observed on day 7 which resolved within 24 hours. An additional 150 mg/kg dose was given on day 8 to evaluate drug-related GI toxicity. Again, vomiting and transient thrombocytopenia were observed with this single dose re-challenge with drug. Following a 26-day washout period, the animal was again dosed for 5 consecutive days with 100mg/kg/day IV bolus on days 34 through 38. Vomiting was observed following the first three doses of study drug. A yellow/brown pigment was identified in the liver and kidney tissue of this animal, but otherwise, there were no adverse morphologic or clinical chemistry alterations noted.

Study 8-ClA-03:

This was a multiple dose study in mice. There were 4 dose groups as follows: 0, 150, 250 and 350 mg/kg IV daily for 5 consecutive days. These doses are equivalent to 0,462,770, 1079 mg/m^2. There were 20 mice in each dose group, 5 of each sex were sacrificed the day after the last dose (acute) and on day 32 (recovery). Clinical signs observed during the dosing period included decreased activity, hunched posture, dyspnea, ruffled hair coat, and sloughing of the skin over the tail. There was a dose-dependent decrease in body weight by the end of day 4 in both male and female mice. The only dose-related alteration in clinical pathology parameters was elevation of BUN in 3 of 5 mice in group 4 (350mg/kg) on study day 5, and this correlated with sever renal lesions. This toxicity was less evident in female mice.

By recovery day 32, no male or female mice had alterations above reference range in BUN value. No drug related gross pathologic findings or effects on organ weights were observed. This study demonstrated that the major targets for acute (day 5) toxicity of 8-chloro-adenosine were kidney, large bowel, pancreas, and thymus. These lesions resolved by day 32 recovery.

Study 8-CIA-04:

This was a multiple dose toxicity study of 8-chloro-adenosine in beagle dogs. There were three dose groups in this study as follows: 0, 6, 18 mg/kg/dose IV bolus daily for 5 consecutive days. These doses are equivalent to 0, 111, and 333mg/m^2. The proposed starting dose in the Phase I part of this clinical trial is

100 mg/m^2 IV daily over 1 hr infusion for 5 consecutive days. There were 10 dogs in each group, three animals of each sex were sacrificed on the day following the last dose (acute) and two animals of each sex were sacrificed on day 32 (recovery) to evaluate for toxicities. There were no early deaths in this study. Clinical observations noted during this study were minimal and included agitation during infusion in a few male animals. Agitation and vomiting were noted in more than one animal but were though related to stress since this occurred in the control group as well. There were no test article-related gross pathologic findings and microscopic evaluation revealed lesions that may be related to test article in the ovaries of these animals. These lesions consisted of follicular or luteal cysts and were present in the animals evaluated for acute toxicities as well in the recovery animals. None of the dogs had atrophy of the renal tubules which was the recovery lesion associated with toxicity of the drug in mice.

2.6 Clinical Studies

A Phase I trial of 8-chloro-adenosine for previously treated chronic lymphocytic leukemia (CLL) was conducted at MD Anderson Cancer Center (NCT00714103). The availability of new anti-CD20 monoclonal antibodies, Bruton's tyrosine kinase inhibitors, PI3 kinase inhibitors and BCL-2 inhibitor therapy resulted in slower than anticipated accrual to the study; the trial was closed to further accrual before the maximum tolerated dose was determined.

Eleven participants enrolled to the study, ten of whom received 8-chloro-adenosine over four dose levels. 8-chloro-adenosine was administered over 1 hour daily for 5 days with cycles repeated every 4 weeks. Table 2.6 summarizes the patient experience on this phase I trial.

Table 2.6 Summary of CLL Patients Treated with 8-Chloro-Adenosine (N=10)

Pt. #	Dose[a] (mg/m^2)	Cycles administered	Reason Off Treatment[b]	Grade 3 or 4 Toxicities Present During Treatment[c]	
				Hematologic	Non-Hematologic
1	45	2	No response	Neutropenia (G4)	-
2	45	1	PD	-	-
3	45	4	PD	Neutropenia (G4) Thrombocytopenia (G3)	-
4	67.5	4	PD	Neutropenia (G4) [d]	Hyperglycemia (G3)
5	67.5	1	PD	Neutropenia (G4) [e]	Fatigue (G3) Abd./Back Pain (G3)
6	67.5	1	PD	Neutropenia (G4)	Fatigue (G3)
8	101.25	1	PD	Neutropenia (G4) Thrombocytopenia (G3)	-
9	101.25	2	No response	Anemia (G3) Neutropenia (G3) [d, e]	-
10	101.25	2	No response	-	-
11	151.88	2	Not available	-	Hypercalcemia (G4) [d]

a- Participants received 8-chloro-adenosine days 1-5 of a 28 day cycle

b- PD: disease progression

c- Grade 3 and 4 toxicities present during treatment **independent of attribution** to 8-chloro-adenosine

d- Highest toxicity was seen cycle 2; absence of superscript "d" indicates highest toxicity was seen in cycle 1

e- Unchanged from baseline value

No serious adverse events were reported. All toxicities seen in this heavily pre-treated patient population were consistent with the underlying disease or the patient's individual clinical history (e.g. hyperglycemia).

A complete list of toxicities by participant in this Phase I Study is provided in a supplement to the protocol. Toxicities present at least two grades above baseline in this population are detailed in Section 6.1.2.

Laboratory Endpoints and Biomarker Investigations:

All patients agreed for pharmacological endpoints and were studied for plasma, cellular, and urine pharmacokinetic and pharmacodynamic endpoints and biomarker analyses. From almost all patients, 17 peripheral blood samples and 4 urine samples were collected during 5-days of therapy.

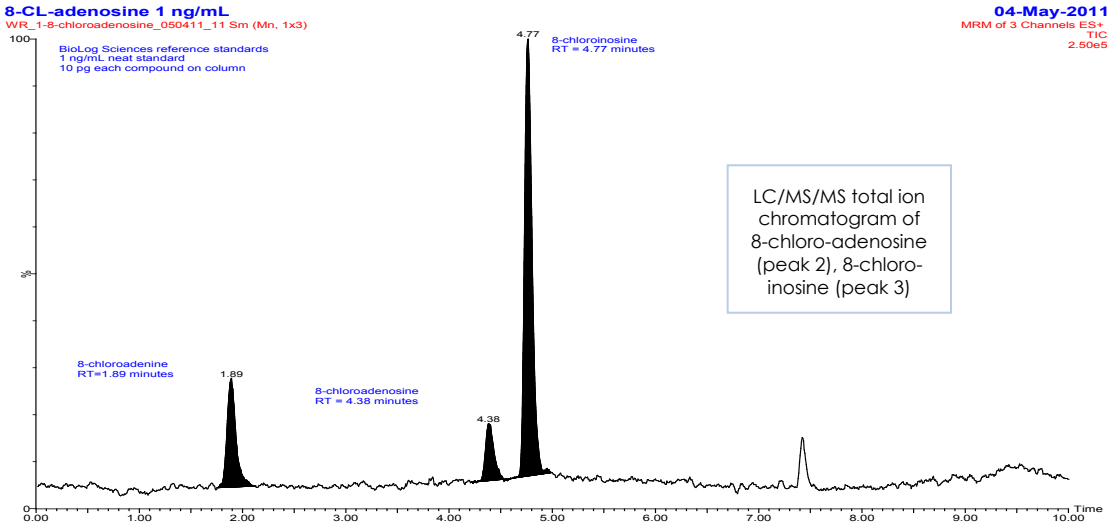
For plasma and urine pharmacokinetic studies

This was done in collaboration with the pharmaceutical development Center at MD Anderson Cancer Center. A new LC/MS assay was developed and validated. This assay identifies and quantitates not only 8-chloro-adenosine but its metabolites such as 8-chloro-adenine and 8-chloro-inosine.

8-chloro-adenosine LC/MS/MS Analytical Method Development

To prepare for quantification of human plasma samples during this Phase I trial, a sensitive and selective LC/MS/MS method for the detection and quantification of 8-chloro-adenosine, 8-chloro-inosine and 8-chloro-adenine has been established using reference standards purchased from BioLog Life Science Institute. Each compound was solubilized according to directions provided by BioLog Science and separate 1 mg/mL (wt/vol) stock solutions were prepared. The stock solutions were then used in establishing the mass spectrometric conditions for the assay. After establishing the MS/MS conditions these parameters were used in setting up the LC/MS/MS analytical method. Initial results with neat reference standards dissolved in acidified water have a lower limit of detection of 1 ng/mL or 1 part per billion with an injection amount of 10 picograms for each of the three compounds (Figure 1). The dynamic range of the assay is between 3 – 4 orders of magnitude. The run time of the analysis is 10 minutes. The instrumentation for this assay utilizes a Waters QuattroUltima mass spectrometer coupled to an Agilent 1100 HPLC. The analytical column used is a Phenomenex Kinetex 2.6 µ C18 2x100 mm, packed with porous outer shell silica particles and a solid inner core. This type of silica has improved chromatographic resolution of the individual compounds, decreased initial observed column carry-over problems and reduced the assay run time from 15 to 10 minutes. The sensitivity and selectivity of this method will be suitable for analysis of parent compound and these metabolites in human biomatrices.

Figure 2.6 Chromatogram of 8-Chloro-adenosine, 8-Chloro-inosine, and 8-Chloro-adenine



Using this sensitive and validated assay, patient samples were analyzed.

Cellular pharmacokinetic studies

For cellular pharmacokinetic analyses, all nine patients' blood samples were collected and analyzed for 8-Cl-ATP. To fully assess intracellular pharmacokinetics and metabolism of 8-chloro-adenosine, we measured 8-Cl-ATP in mononuclear cells isolated from patients prior to and following infusion. Typically, the mononuclear cell population in patients with CLL consists of 95% or greater leukemia cells. In general, there was dose-dependent increase in intracellular concentration of 8-Cl-ATP. 8-Cl-ATP accumulates at a fast rate and to a detectable level, and 8-Cl-ATP is maintained in the cell for at least 24 hours, In addition, for each patient, with every infusion, there was an incremental increase in the level of 8-Cl-ATP. This was specifically due to the fact that there was minimal decrease in 8-Cl-ATP levels after end of infusion indicating that this triphosphate is long-lived in cells.

Cumulative accumulation of 8-Cl-ATP is clearly visible when accumulation of 8-Cl-ATP is plotted against time of drug infusion. This is consistent with our previous in vitro investigations in CLL cells (Balakrishnan et al. Blood 2005) which that 8-Cl-ATP is long-lived in cells. In parallel to the accumulation of 8-Cl-ATP, there was a decline in the ATP pool during in vitro investigations. For those studies, we incubated cells with 10 µM 8-chloro-adenosine for up to 24 hours. This resulted in more than 300 uM 8-Cl-ATP.

8 chloro-adenosine was prepared for clinical protocol 2004-0144 by the MD Anderson Cancer Center pharmacy per protocol specifications. Prior to release for use in humans, drug was tested for sterility and endotoxin by Celsis Analytical Services in St. Louis, MO. To support clinical use, each lot produced approximately 100 vials. The shelf-life stability study included testing each lot at 0, 1, 3, 6, 9, 12, 18, and 24 month time points. 3 separate lots were produced to establish shelf-life stability and for clinical use. In the 1st lot (C040408-13), 96 vials were produced, with 9 of these developing crystals or precipitation. In lot 2 (C051409-10), 99 vials were produced, with 9 developing precipitation. The final lot (C111809-01) produced 100 vials, of which 28 precipitated. Given the number of vials that precipitated, and especially the rapidity of the precipitation in the 3rd batch, it was determined that 10 mg/ml exceeded maximum solubility. Stability data were communicated to the principal investigator during the study.

3.0 PATIENT ELIGIBILITY

3.1 Inclusion Criteria

Participants must meet the following criteria on screening examination to be eligible to participate in the study:

Patient MRN:	Patient Initials (F, M, L):

Informed Consent

___ 1. All subjects must have the ability to understand and the willingness to sign a written informed consent.

Age Criteria, Performance Status, and Life Expectancy

___ 2. Patients must be age ≥ 18 years.

___ 3. Patients must have a life expectancy of > 3 months.

___ 4. Patients must exhibit an ECOG performance status of 0-2.

Nature of Illness and Treatment History

___ 5. Patients must have a diagnosis of AML as per WHO Classification of Hematologic Neoplams.

___ 6. Patients must meet one of the three treatment history criteria:

- Relapsed AML who have failed at least 1 line of salvage therapy
- De novo AML who have not achieved CR after 2 lines of therapy
- AML evolving from MDS or myeloproliferative disorder who have failed hypomethylating agent or induction chemotherapy
- Patients who have relapsed after allogeneic HCT are eligible if they are at least 3 months after HCT, do not have active GVHD and are off immunosuppression except for maintenance dose of steroids (prednisone 10 mg/day or less).

___ 7. At least 2 weeks from prior chemotherapy or radiation therapy to time of start of treatment, except for hydroxyurea or corticosteroid therapy which may be continued through Cycle 1.

Clinical laboratory parameters

___ 8. AST and ALT ≤ 2.5 x ULN and total bilirubin ≤ 1.5 X ULN

___ 9. QTc ≤ 480 ms

___ 10. Calculated creatinine clearance (CrCl) ≥ 50 mL/min per 24 hour urine collection or the Cockcroft-Gault formula

$$\text{CrCl (mL/min)} = \frac{\text{(140-age) x actual body weight (kg)}}{\text{72 x serum creatinine (mg/dL)}} \text{ (x 0.85 for females)}$$

Or

$$\text{CrCl (mL/min)} = \frac{\text{(140-age) x actual body weight (kg)}}{\text{0.8136 x serum creatinine (umol/L)}} \text{ (x 0.85 for females)}$$

___ 11. Negative serum or urine β-HCG test (female patient of childbearing potential only), to be performed locally within the screening period.

Child Bearing Potential

___ 12. Agreement by females of childbearing potential and sexually active males to use an effective method of contraception (hormonal or barrier method of birth control or abstinence) prior to study entry and for three months following duration of study participation. The effects of study treatment on a developing fetus have the potential for teratogenic or abortifacient effects. Should a woman become pregnant or suspect that she is pregnant while participating on the trial, she should inform her treating physician immediately.

3.2 Exclusion Criteria

Prospective participants who meet any of the following criteria will not be eligible for admission into the study:

Concomitant medications

___ 13. Current or planned use of other investigational agents, or concurrent biological, chemotherapy, or radiation therapy during the study treatment period. (See inclusion criterion #7 for washout times).

___ 14. Expected to undergo HCT within 120 days of enrollment.

___ 15. Current or planned use of agents that prolong or suspected to prolong QTc

Other illnesses or conditions

___ 16. Diagnosis of acute promeylocytic leukemia

___ 17. Active central nervous system leukemia

___ 18. Active fungal infection or bacterial sepsis

___ 19. Active peptic ulcer disease

___ 20. History of heart failure or cardiac arrhythmia

___ 21. Other active malignancy except for localized skin cancer, bladder, prostate, breast or cervical carcinoma in situ.

___ 22. Pregnant women and women who are lactating. 8-chloro-adenosine is an agent with the potential for teratogenic or abortifacient effects. Because there is an unknown but potential risk for adverse events in nursing infants secondary to treatment of the mother with 8-chloro-adenosine, breastfeeding should be discontinued if the mother is treated with 8-chloro-adenosine.

___ 23. Any other condition that would, in the Investigator's judgment, contraindicate the patient's participation in the clinical study due to safety concerns or compliance with clinical study procedures

Noncompliance

___ 24. Prospective participants who, in the opinion of the investigator, may not be able to comply with all study procedures (including compliance issues related to feasibility/logistics).

3.3 Inclusion of Women and Minorities

The study is open anyone regardless of gender or race/ethnicity. Efforts will be made to extend the accrual to a representative population, but in a trial which will accrue approximately 43 subjects, a balance must be struck between subject safety considerations and limitations on the number of individuals exposed to potentially toxic or ineffective treatments on the one hand and the need to explore gender, racial, and ethnic aspects of clinical research on the other. If differences in outcome that correlate to gender, racial, or ethnic identity are noted, accrual may be expanded or additional studies may be performed to investigate those differences more fully.

4.0 SCREENING AND REGISTRATION PROCEDURES

4.1 Screening Procedures

Diagnostic or laboratory studies performed exclusively to determine eligibility for this trial will be done only after obtaining written informed consent. Studies or procedures that were for clinical indications (not exclusively to determine study eligibility) may be used for baseline values, even if the studies were done before informed consent was obtained. Reference is made to Section 10.0 – Study Calendar.

4.2 Informed Consent

The investigational nature and objectives of the trial, the procedures and treatments involved and their attendant risks and discomforts, and potential alternative therapies will be carefully explained to the subject and a signed informed consent will be obtained. Documentation of informed consent for screening will be maintained in the subject's research chart and medical record and the patient will receive a copy of the signed informed consent document. All Institutional, Federal, and State regulations concerning Informed Consent will be fulfilled.

4.3 Registration Requirements/Process

Confirming and reserving a slot

Eligible subjects will be registered on the study centrally by the Data Coordinating Center (DCC) at City of Hope. Staff (including physicians, protocol nurses and/or CRCs) should call the DCC at (626) 256-4673, ext. 64267 to verify the current dose level and slot availability, and to reserve a slot for a specific prospective subject. Slots can only be held for a limited time.

Eligible subjects must be registered **prior** to start of protocol therapy. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a subject does not receive protocol therapy following registration, the subject's registration on the study may be canceled after discussion with the PI. The Data Coordinating Center should be notified of cancellations as soon as possible.

Registration Process – City of Hope Subjects

Once a slot at a dose level has been reserved, the signed informed consent has been obtained, all pretreatment evaluations have been performed, and subject's eligibility has been confirmed by the Data Coordinating Center, a subject will be registered on study.

To register a subject, the treating physician should contact the protocol nurse or the responsible Clinical Research Coordinator (CRC) in the Clinical Trial Office (CTO) to complete the eligibility checklist.

The protocol nurse or CRC will contact the Data Coordinating Center at City of Hope (626-256-4673, ext. 64267 or via e-mail at dcc@coh.org), scan and EMAIL a copy of the completed and signed eligibility checklist, a copy of the signed Informed Consent (including a copy of signed subject's bill of Rights and HIPAA authorization form) and copies of any required pre-study test results that are not readily available in the COH electronic medical record to dcc@coh.org.

The protocol nurse or CRC may then call the Data Coordinating Center (626-256-4673 ext. 64267) to confirm receipt of all registration documents. To complete the registration process, the Data Coordinating Center will:

- Verify and confirm the subject's eligibility.
- Assign a subject accession number (for example, COH-001, COH-002, etc.).
- Register the subject on study centrally (the City of Hope CRC assigned to the trial will still be responsible for accessioning via MIDAS).
- Assign the subject to a dose level (as applicable).
- Complete and email a Confirmation of Registration form within 24 hours to include the COH subject study number and dose level to the study team, which will include the Principal Investigator, treating physician, protocol nurse, CRC and COH IDS Pharmacy.
- Call the protocol nurse and/or CRC to verbally confirm registration.
- Enter the subject into Medidata RAVE.
- A subject failing to meet all protocol requirements will not be registered.

Patients must begin protocol treatment within 1 week following registration.

4.4 Screen Failures and Registered Participants Who Do Not begin Study Treatment

The reason for screen failure or failure to begin treatment for registered participants will be documented.

4.5 Dose Level Assignment

During the phase I portion of the trial, participants will be assigned to the dose level that is currently open. Participants in the phase II portion of the trial will initiate study agent at the recommended phase II dose level.

5.0 TREATMENT PROGRAM

5.1 Treatment Overview

This is a Phase I/II, open label, non-randomized, dose escalation study to determine the MTD, safety, preliminary efficacy and pharmacokinetics and pharmacodynamics of 8-Cl-A administered as a 4 hour intravenous infusion daily for 5-days every 4 weeks in patients with relapsed/refractory AML. Participants will receive up to four cycles of treatment. Participants will be hospitalized during treatment administration (at minimum the first 6 days of the cycle). Participants will be admitted to the telemetry unit and undergo continuous cardiac monitoring from start of treatment until 24 hours after completion of therapy during Cycles 1-4.

Participants who end study treatment for reasons other than disease progression will undergo active follow-up (Section 5.9) per institutional SOP for response assessment until disease progression or the initiation of a new therapy. All participants will be followed for survival (Section 5.9).

For a detailed tabular view of the treatment, monitoring, and follow-up schedule, see the Study calendar in Section 10.

5.2 Treatment cycle definition

Participants will be treated in 28-day treatment cycles until disease relapse, progression, unacceptable toxicity, withdrawal of consent, or other protocol specified parameters to stop treatment.

Once a treatment cycle has began (i.e. the Day 1 dose administered) if there is a hold in administering the study agent (see section 6.3.2), **missed doses may be made up if the criteria to resume treatment are met within 48 hours, or by Day 5 of the treatment cycle, whichever is later**. Otherwise missed administrations will not be made up, and treatment will resume with the next cycle (assuming start of cycle criteria are met). Should a hold or delay in treatment occur, after the last dose is administered in a given cycle, there will be a 21 day break, which may result in a delay in the start of the subsequent cycle.

5.3 Phase I Treatment Plan

The phase I trial will implement a 3+3 dose escalation, de-escalation design to ensure safety and to allow for the evaluation of toxicities associated with 8-chloro-adenosine. See Section 13.3 for dose escalation/de-escalation rules, and Section 13.2 for the definition of DLT.

For this single agent phase I trial, up to four dose levels ('a' levels) of 8-chloro-adenosine will be considered if the accelerated stage is maintained throughout. The first cohort will start at dose level 1a. Intermediate dose levels, in increments of 50% from the planned four dose levels, will be explored in the presence of dose limiting toxicity or unacceptable moderate toxicity (see Section 13.2). Intermediate dose levels ('b' levels) are defined as follows: 150mg/m^2; 300 mg/m^2; and 600 mg/m^2.

Table 5.3.1 Phase I Dose Levels

	Dose Level	8-Chloro-Adenosine Dose
	-1	50 mg/m^2 Days 1-5 of a 28 day cycle
	1a	100 mg/m^2 Days 1-5 of a 28 day cycle
	1b	150 mg/m^2 Days 1-5 of a 28 day cycle
	2a	200 mg/m^2 Days 1-5 of a 28 day cycle
	2b	300 mg/m^2 Days 1-5 of a 28 day cycle
Starting dose with amendment v07	**3a**	**400 mg/m^2 Days 1-5 of a 28 day cycle**
	3b	600 mg/m^2 Days 1-5 of a 28 day cycle
	4a	800 mg/m^2 Days 1-5 of a 28 day cycle

There will be no intra-patient dose escalation; participants will undergo dose modification due to toxicity per Section 6.3.

5.4 Phase II Treatment Plan

Following the phase I trials described above, a single arm phase II study will be conducted where the recommended phase II dose (RP2D) is brought forward for activity evaluation.

There will be no intra-patient dose escalation; participants will undergo dose modification due to toxicity per Section 6.3.

5.5 Agent Administration

8-Chloro-adenosine will be administered as an intravenous infusion daily for the first 5 days of each cycle, based on the BSA calculated from the weight measurement taken on Day 1 of the treatment cycle. See Section 8.1.4 for agent preparation. 8-chloro-adenosine will be infused over 4 hours +/- 10 minutes. The infusion rate should not exceed 500 ml/hr. Infusion line will be flushed with 5% dextrose after completion of infusion. Infusion is to be completed within 12 hours of agent preparation during which time it should be stored at room temperature.

Start of cycle criteria are listed in Section 6.2.

Dose levels for dose modification are detailed in Table 5.3.1. There will be no intra-patient dose escalation; participants will undergo dose modification due to toxicity per Section 6.3.2.

5.6 Study Procedures

For a detailed list of all study procedures including timing and windows, see Section 10.

5.7 Duration of Therapy & Criteria for Removal from Study Treatment

Duration of therapy will depend on individual response, evidence of disease progression and tolerance, or completion of 4 cycles of study treatment. Patients may continue for up to 4 cycles of treatment unless unacceptable toxicity or disease relapse/progression. In the absence of treatment delays due to adverse events, treatment may continue until one of the following criteria applies:

- Participant demonstrates disease progression.
- Intercurrent illness that prevents further administration of treatment
- Unacceptable adverse event(s) (see Section 13.2, 6.3.1)
- Participant withdraws from the treatment phase of the study
- General or specific changes in the participant's condition that render the participant unacceptable for further treatment in the opinion of the treating investigator.

Documentation of the reason for discontinuing therapy and the date effective should be made in the medical record and appropriate eCRF. The participant should then proceed to off-treatment procedures for safety monitoring and follow-up procedures. The participant status is to be modified in the MIDAS system once the off-treatment period is completed. Alternative care options will be discussed with the participant.

5.8 End of Treatment Evaluations

All participants will be followed until resolution or stabilization of any serious adverse events occurring during treatment or starting within 30 days of last study drug or completion of the Day 30 End of Treatment assessments. See Section 10 for a list of all assessments and windows.

5.9 Active Follow Up and Survival Follow Up

Participants who do not progress while on treatment will be in *Active Follow Up*, where they will continue to undergo response assessment until disease progression, the initiation of a new therapy or for a maximum of 2 years after their first dose of study agent.

Participants who progress during Treatment Phase skip *Active Follow-Up* phase and continue directly into *Survival Follow-up* phase.

In *Survival Follow-Up* participants will be followed for survival information for a maximum of 2 years after their first dose of study agent. This may be obtained by reviewing the City of Hope medical record,

contacting the participant and/or review of outside medical records. Note: This is not intended to be an all inclusive list.

The schedule and windows for assessments and data collection points are further detailed in Table 10.

5.10 Criteria for Completion of Study Participation

Participants will be followed until disease progression, unacceptable toxicities, patient refusal of further protocol therapy or for a maximum of 2 years after their first dose of study agent. Participants may also be removed if in the investigator's medical judgment, further participation would be injurious to the health and well being of the patient, or is not in the best interest of the patient.

The reason for study removal and the date the participant was removed must be documented in the source documentation and the study-specific case report form (CRF). The participant's status is to be modified in the MIDAS system once the participant completes the study.

Efforts will be made to obtain an autopsy on patients who die. Careful evaluation of potential target organs for toxicity will be made.

5.11 Supportive Care, Other Concomitant Therapy, Prohibited Medications

Participants will receive supportive care as clinically appropriate. This includes red cell and platelet transfusion support, antibiotics, antifungals and antiviral agents and agents for prophylaxis and management of tumor lysis. Hydroxyurea may be used at the treating physician's discretion during Cycle 1 of the Phase I (dose escalation) part of the protocol in order to control blood counts. Leukapheresis is permitted during Cycle 1 of Phase I to manage symptomatic leukostasis. All patients will receive oral metoprolol 25mg twice daily starting the day before initiation of Cycle 1 until 24 hours after completion of therapy.

Prohibited therapies from Cycle 1 Day 1 until 30-days post-last dose: Other anti-neoplastic therapies, agents that prolong or suspected to prolong QTc, and other investigational agents.

Patients must be instructed not to take any additional medications (including over-the-counter products) during the trial without prior consultation with the investigator. All medications taken within 28 days of planned start of treatment should be recorded. If concomitant therapy must be added or changed, the reason and name of the drug/therapy should be recorded.

5.12 Additional Studies

See Section 9.0 for correlative studies.

6.0 ANTICIPATED TOXICITIES, START OF CYCLE CRITERIA, AND DOSE DELAYS/MODIFICATIONS

6.1 Toxicities to 8-Chloro-adenosine

6.1.1 Expected (known) toxicities to 8-chloro-adenosine

To date no toxicities have been attributed to 8-chloro-adenosine in humans.

6.1.2 Possible anticipated toxicities to 8-chloro-adenosine

Events seen in chronic lymphocytic leukemia patients regardless of attribution that were at least two grades over baseline are listed with the highest grade indicated:

Hematologic: anemia (G3), neutropenia (G4), thrombocytopenia (G3)

Gastrointestinal: abdominal pain (G3), diarrhea (G2),

Metabolic: hypercalcemia (G4), hyperglycemia (G3), hypouricemia (G2),

General/Other: back pain (G3), fever (G2), sunburn (G2), flu syndrome (G2)

Toxicities seen in animal studies (murine and canine) using a range of regimens and doses (Section 2.5) are as follows:

Hematologic: transient thrombocytopenia

Gastrointestinal: vomiting

Renal: elevated BUN, renal nephrosis

General/Other: decreased activity, sloughing of the skin, dyspnea

Toxicities which may be foreseeable based on toxicities seen with other adenosine analogues (Cladribine, Didanosine, Fludarabine, Tenofovir) include the following (note: the asterisk indicates extremely rare but serious events):

Hematologic: thrombocytopenia, neutropenia, anemia, myelosuppression, pancytopenia*

Dermatologic: pruritus, rash, Stevens-Johnson syndrome*, toxic epidermal necrolysis*

Gastrointestinal: vomiting, nausea, diarrhea, abdominal pain, mucositis, stomatitis, anorexia, increased serum amylase, pancreatitis

Hepatic: increased liver enzymes, liver failure*

Metabolic: hyperuricemia

Musculoskeletal: arthralgia, myalgia, bone pain, backache, muscle weakness

Neurologic: headache, peripheral neuropathy, dizziness, insomnia

Renal: increased serum creatinine, renal failure*

Other: anaphylactoid reaction*, alopecia, fatigue, fever, chills, infection, pain, dyspnea, edema, diaphoresis, phlebitis

6.2 Start of Cycle Criteria:

Hematologic toxicities:

- For participants with CR, all hematologic toxicities must resolve to ≤ Grade 2
- For participants without CR, hematologic toxicity maybe of any grade.

Non-hematologic toxicities:

- Non-hematologic toxicities related to study agent must resolve to ≤ Grade 2.
- Non-hematologic toxicities not considered related to study agent may delay of start of cycle criteria per the discretion of the treating investigator.

For holds due to toxicities related to study agent, if the participant does not meet criteria to resume treatment by Day 49 from start of cycle, the participant must permanently discontinue study treatment.

See Section 6.3 for dose modifications due to toxicity.

6.3 Dose Modifications of 8-Chloro-adenosine

6.3.1 General Information

a. The study will use the NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.03 to grade toxicities. A copy of the version 4.0 can be downloaded from:

http://evs.nci.nih.gov/ftp1/CTCAE/About.html

b. Intra-patient dose escalation is never permitted in this study. Rules for dose modification for are found in Table 6.3.2.

c. **For treatment holds if the participant does not meet criteria to resume treatment by Day 49 from start of cycle, the participant must permanently discontinue study treatment.**

d. When dose reductions are applicable, the dose will resume at the next lowest safe dose. Dose levels including intermediate dose levels for dose reduction are detailed in Table 5.3.1.

6.3.2 Dose Modifications and Treatment Interruptions

The table below details the specific dose modifications and treatment interruptions for administration of 8-chloro-adenosine; they are to be used in agreement with the information in Section 6.3.1. Unless listed in Table 6.3.2, treatment will continue without interruption or dose modification. Start of cycle criteria will apply to initiate a new cycle of therapy (Section 6.2).

Table 6.3.2 Dose Modifications and Interruptions to 8-Chloro-adenosine Administration*

Adverse Event	Treatment Modification
Non-Hematologic toxicities considered **RELATED** to 8-chloro-adenosine in all participants:	
Grade 3 toxicity **except** for the following: • vomiting, nausea, diarrhea that responds to maximum medical therapy • electrolyte imbalance, fatigue, anorexia, asthenia, stomatitis, mucositis, infection, dermatitis	Hold study treatment until resolution to ≤ Grade 2. Resume at pre-hold dose.
Grade 4 toxicity:	Hold study treatment until resolution to ≤ Grade 2. Resume at 1 dose level reduction unless the toxicity is a grade 4 electrolyte imbalance correctable within 48 hours or grade 4 vomiting that responds to maximum medical therapy within 48 hours. In such cases patients can resume treatment at the assigned dose without reduction at the Physician's discretion.
Hematologic toxicity **RELATED** to 8-chloro Adenosine	
Grade ≥3 peripheral blood cytopenia not resolving to ≤Grade 2 by day 49 from start of cycle in patients who have less than 5% bone marrow blasts	No additional cycles of study drug will be administered
Other unspecified **Non-Hematologic** Toxicities considered **UNRELATED** to study agent	
Other unspecified events of any grade considered unlikely or not related to study agent.	Maintain treatment with study agent. Interruption of study treatment is permitted if the investigator consults with the Principal Investigator to determine that this is in the best interest of the participant.

*: See section 5.2 for additional criteria to resume, make up missed doses.

7.0 DATA AND SAFETY MONITORING PLAN

7.1 Definition of Risk Level

This is a Risk Level 4 study as defined in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. This determination was made because the study involves COH as IND holder and administering 8-chloro-adenosine as a single-dose agent.

7.2 Monitoring and Personnel Responsible for Monitoring

The Protocol Management Team (PMT) is responsible for monitoring the data and safety of this study. The PMT consists of the Principle Investigator (PI), Co-Investigator(s), Biostatistician, Research Protocol Nurse, and Clinical Research Coordinator. The PMT is required to submit periodic status reports (i.e., the PMT Report) according to the frequency prescribed in the City of Hope Institutional Data and Safety Monitoring Plan [policy dated 07/09/2014]. Important decisions made during PMT meetings (i.e., dose escalation, de-escalation, etc.) only need to be noted in the PMT Report submitted to the Data and Safety Monitoring Committee (DSMC).

Dose Escalation: This study will utilize the Phase I tracking log to monitor data and safety for dose escalation. The tracking log will contain dose levels administered, dose limiting toxicities (DLT), DLT-defining adverse events, and any details regarding dose escalation. The record of doses administered and resultant adverse events will be included in the PMT report. **Adverse Events and Serious Adverse Events**

The PI will be responsible for determining the event name, assessing the severity (i.e., grade), expectedness, and attribution of all adverse events.

Adverse Event (AE) - An adverse event is any untoward medical experience or change of an existing condition that occurs during or after treatment, whether or not it is considered to be related to the protocol intervention.

Reporting Non-serious Adverse Events – Adverse events will be collected after the patient is given the study treatment or any study related procedures. Adverse events will be monitored by the PMT. Adverse events that do not meet the criteria of serious OR are not unanticipated problems will be reported only in the PMT Report.

Serious Adverse Event (SAE) [Modified from the definition of unexpected adverse drug experience in 21 CFR 312.32] - defined as *any expected* or *unexpected adverse event*s that result in any of the following outcomes:

- Death
- Is life-threatening experience (places the subject at immediate risk of death from the event as it occurred)
- Unplanned hospitalization (equal to or greater than 24 hours) or prolongation of existing hospitalization
- A persistent or significant disability/incapacity
- A congenital anomaly/birth defect
- Secondary malignancy
- Any other adverse event that, based upon appropriate medical judgment, may jeopardize the subject's health and may require medical or surgical intervention to prevent one of the outcomes listed above (examples of such events include allergic bronchospasm requiring intensive treatment in the emergency room or at home, blood dyscrasias of convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse).

Reporting Serious Adverse Events - begins after study treatment or any study related procedures. All SAEs occurring during this study, whether observed by the physician, nurse, or reported by the patient, will be reported according to the approved City of Hope's Institutional policy [policy effective date: 05/14/14]. Serious Adverse Events that require expedited reporting will be submitted electronically using iRIS.

Adverse Event Name and Severity

The PI will determine the adverse event name and severity (grade) by using the CTCAE version 4.03.

Expected Adverse Event - Any event that does not meet the criteria for an unexpected event, OR is an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Unexpected Adverse Event [21 CFR 312.32 (a)] – An adverse event is unexpected if it is not listed in the investigator's brochure and/or package insert; is not listed at the specificity or severity that has been observed; is not consistent with the risk information described in the protocol and/or consent; is not an expected natural progression of any underlying disease, disorder, condition, or predisposed risk factor of the research participant experiencing the adverse event.

Adverse Event Attribution

The following definitions will be used to determine the causality (attribution) of the event to the study agent or study procedure. **With Amendment v09:** A two level toxicity attribution scale will be used. In terms of attribution, all recorded adverse events will be classified as either related or unrelated to the study drug. Prior to this amendment a five level attribution scale (definite, possible, probable, unlikely, unrelated) was used.

Related -All adverse events will be considered relevant to determining dose-limiting toxicities and reporting unless the event can clearly be determined to be unrelated to the drug.

Unrelated -The AE is clearly not related to the investigational agent or study procedure and is attributable to another cause(s).

COH Held IND

Serious Adverse Events meeting the requirements for expedited reporting to the Food and Drug Administration (FDA), as defined in 21 CFR 312.32, will be reported as an IND safety report using the MedWatch Form FDA 3500A for Mandatory Reporting.

The criteria that require reporting using the Medwatch 3500A are:

- Any unexpected fatal or life threatening adverse experience associated with use of the drug must be reported to the FDA no later than 7 calendar days after initial receipt of the information [21 CFR 312.32(c)(2)]
- Any adverse experience associated with use of the drug that is both serious and unexpected must be submitted no later than 15 calendar days after initial receipt of the information [21 CFR 312.32(c)(1)]
- Any follow-up information to a study report shall be reported as soon as the relevant information becomes available. [21 CFR 312.32(d)(3)]

The PI or designee will be responsible for contacting the Office of IND Development and Regulatory Affairs (OIDRA) at COH to ensure prompt reporting of safety reports to the FDA. OIDRA will assist the PI with the preparation of the report and submit the report to the FDA in accordance with the approved City of Hope's Institutional policy [policy effective date: 05/14/14].

Deviations and Unanticipated Problems

Deviation - A deviation is a divergence from a specific element of a protocol that occurred without prior IRB approval. Investigators may deviate from the protocol to eliminate immediate hazard(s) for the protection, safety, and well-being of the study subjects without prior IRB approval. For any such deviation, the PI will notify the COH DSMC and IRB within 5 calendar days of its occurrence via iRIS in accordance with the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11].

Single Subject Exception (SSE)

An SSE is a planned deviation, meaning that it involves circumstances in which the specific procedures called for in a protocol are not in the best interests of a specific patient. It is a deviation that is anticipated and receives prior approval by the PI and the IRB. The SSE must be submitted as a "Single Subject Exception Amendment Request" via iRIS in accordance with IRB guidelines and the Clinical Research Protocol Deviation policy [policy effective date: 11/07/11]. An IRB approved SSE does not need to be submitted as a deviation to the DSMC.

Unanticipated Problem (UP) – Any incident, experience, or outcome that **meets all three** of the following criteria:

1. Unexpected (in terms of nature, severity, or frequency) given the following: a) the research procedures described in the protocol-related documents such as the IRB approved research protocol, informed consent document or Investigator Brochure (IB); and b) the characteristics of the subject population being studied; **AND**
2. Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcomes may have been caused by the drugs, devices or procedures involved in the research); **AND**
3. Suggests that the research places subjects or others at greater risk of harm (including physical, psychological, economic, or social harm) than previously known or recognized.

Any UP that occurs during study conduct will be reported to the DSMC and IRB in accordance with the City of Hope's Institutional policy [policy effective date: 05/14/14] using iRIS.

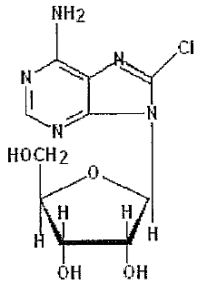
COH Held IND

The Office of IND Development and Regulatory Affairs (OIDRA) will assist the PI in reporting the event to the Food and Drug Administration (FDA).

8.0 AGENT INFORMATION

8.1 8-Chloro-adenosine

8.1.1 Description and classification



Chemical Name: 8-chloro-adenosine

Other name: NSC 354258

Manufacturer: Active Pharmaceutical Ingredient produced by Ash Stevens. Drug formulation manufactured by the University of Iowa Pharmaceuticals.

8-chloro-adenosine is an adenosine analogue with a ribose sugar and a chlorine group at the 8-position of the adenine base. 8-chloro-adenosine has a molecular formula C[10]H[12]N[5]O[4]Cl and molecular weight of 301.68. The melting point is 198-199°C. It is a white powder that has an intrinsic solubility in water of 4.8 mg/ml. It is stable (no degradation) at 25°C or 35°C for at least 2 months, however, it is unstable in water at pH<3 and pH>8. A 5 mg/ml aqueous solution appears to be stable at pH 7.0 for over 1 year.

8-chloro-adenosine was prepared in a 3-step reaction from adenosine: 1) acetylation of adenosine with acetic anhydride; 2) reaction with m-chloro-pherbenzoic acid/HCl to yield 8-chloro-adenosine triacetate; 3) deacetylation with sodium methoxide/methanol.

The drug is supplied at a concentration of 5 mg/mL in 5% dextrose (20 mL per vial).

8.1.2 Mode of action, pharmacology, toxicology

See Section 2.4.2 for mechanism of action, Sections 2.5 and 2.6 for toxicology, and Section 2.6 for pharmacology.

8.1.3 Storage and stability

Stability testing will be ongoing by the City of Hope Manufacturing Core. The aqueous solution should be maintained at room temperature.

8.1.4 Preparation

Agent preparation will proceed as follows:

- Calculate dose based on assigned dose level and patient's BSA using the body weight obtained on Day 1 of treatment cycle.
- Check vial for particulates. Do not use if there is evidence of particulates.
- Withdraw the calculated dose volume of 8-chloro-adenosine.
- Inject calculated dose via a 0.22 µm filter into 5% dextrose to a concentration not exceeding 2 mg/ml.
- Store prepared agent at room temperature until infusion.

Note: Infusion should be completed within 12 hours of preparation.

8.1.5 Agent administration

8-chloro-adenosine will be infused over 4 hours +/- 10 minutes. The infusion rate should not exceed 500 ml/hr.

Infusion is to be completed within 12 hours of agent preparation.

8.1.6 Accountability

The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of each of the agents using the NCI Drug Accountability Record or another comparable drug accountability form.

9.0 CORRELATIVE/SPECIAL STUDIES

9.1 Pharmacokinetics and Pharmacodynamics

9.1.1 Clinical Pharmacology Objectives

- To describe the plasma, urinary and cellular pharmacokinetics of 8-chloro-adenosine and metabolites.
- To determine the impact of 8-chloro-adenosine on cellular ATP pool in AML blasts.
- To assess the impact of 8-chloro-adenosine therapy on select short-lived mRNAs and corresponding proteins in circulating AML blasts.
- To correlate clinical responses and toxicity with plasma/urine 8-chloro-adenosine level (PK), cellular 8-Cl-ATP (PK) and cellular ATP pool.

9.1.2 Blood Sample Collection

Blood samples will be collected from an indwelling venous catheter or by venipuncture, at a **separate anatomic** site as drug infusion e.g., in the opposite arm of the drug infusion. In cases of poor venous access, specimen may be drawn from a catheter lumen separate from that used for drug infusion after approval by PI. At each time point indicated in Table 9.1.2, peripheral blood will be collected into **three* 7 ml green-top tubes** (sodium or lithium heparin) containing deoxycoformycin to prevent blood clotting and ex vivo deamination of 8-chloro-adenosine. Only one 7ml green top tube need be collected unless circulating blast count is over 5000/µL. Tubes will be **inverted several times** and then **immediately placed on ice** and brought **promptly** (so that the sample can be processed within the hour) to the CICSL (Shapiro rm. 1042) or other processing laboratory. Notify the CICSL (Leslie Smith-Powell or her representative) by e-mail to LSmith-Powell@coh.org in advance of sampling (ideally >1 day in advance).

Table 9.1.2 Time Points of Blood Sample Collection for PK/PD Studies

Pt. MRN:	Pt. Initials (F, M, L):	Pt. Study ID:

	Time Point During Cycle 1	No. of tubes collected*	Time (h:m of 24-hr clock)	Date	Initials
Day 1	0-24hr Pre-infusion				
	Start of Infusion TIME	--			
	Within last 15 minutes of infusion				
	End of Infusion (EOI) TIME#	--			
	1 hr post EOI +/- 15 min				
	3 hr post EOI +/- 15 min				
	6-8 hr post EOI				
	12-18 hr post EOI				
Day 2	0-1 hr pre-infusion				
	Start of Infusion TIME	--			
	Within last 15 minutes of infusion				
	End of Infusion TIME#	--			
Day 3	0-1 hr pre-infusion				
	Start of Infusion TIME	--			
	Within last 15 minutes of infusion				
	End of Infusion TIME#	--			
Day 4	0-1hr Pre-infusion				
	Start of Infusion TIME	--			
	Within last 15 minutes of infusion				
	End of Infusion TIME#	--			
Day 5	0-1hr Pre-infusion				
	Start of Infusion TIME	--			
	Within last 15 minutes of infusion				
	End of Infusion TIME#	--			

*Collect **three tubes if** patient has ≥ 5000 circulating leukemic blasts per micro liter of blood (on a recent clinical lab result (e.g. within 24 hours) and if collection occurs when cell processing is available by CICSL. Otherwise collect only **one tube** and process sample for plasma only (may be done by Clinical Pathology Lab if CICSL not available).

End of infusion time includes flush time.

The biospecimen coordinator for the study can be reached at 626-256-4676 ext. 82157.

9.1.3 Urine Specimen Collection

All urine will be collected in three 8 hr +/-30 min sequential intervals (e.g. 0-8 hrs, 8-16 hrs, and 16-24 hrs) during the first 24 hours after the first dose of cycle 1. Samples will be delivered to CICSL in Shapiro room 1042 at room temperature. Samples may be delivered to CICSL in a single batch.

9.1.4 Sample Processing

Urine

- For urine samples, the total volume will be recorded for each of the 3 collection intervals (0-8 hrs, 8-16 hrs, and 16-24 hrs) and a 10 ml aliquot for each of the three time-points will be stored frozen at < -70^{o}C until shipping.

Plasma

- Sample processing is to start within 1 hour of sample collection.
- For plasma, anti-coagulated whole blood samples (one 7 ml green-top tube) will be processed by centrifugation for 10 minutes at 1500 x g at 4^{o}C. The resulting plasma will be transferred to appropriately labeled polypropylene tubes and frozen at < -70^{o}C until shipping.

PBMCs

- Sample processing is to start within 1 hour of sample collection.
- For leukemic blast isolation, blood samples will be collected from those patients with ≥ 5000 circulating leukemic blasts per microliter of blood and at times when laboratory processing is available. Peripheral blood mononuclear cells (PBMC) will be isolated from whole blood by Ficoll-gradient separation as described:
    - Whole blood (two 7 ml green-top tubes) will be diluted 1:5 with ice cold PBS and carefully layered on top of 10 ml of Histopaque® (Sigma cat. # 1077-1) in 50 ml polypropylene centrifuge tubes.
    - Blood/ficoll tubes will then be centrifuged at 1500 x g for 25 minutes and 4°C with the break in the "off" position. The mononuclear blood cells at the interface between the Histopaque® and the top aqueous layer will be aspirated into a clean 15 ml polypropylene centrifuge tube using a glass Pasteur pipette.
    - The aspirated PBMC will then be washed x 2 in ice cold PBS, cells will be counted, and then divided to perform pharmacodynamic studies and correlative studies as indicated in the table below. In case of limited cell number, PBMC samples for intracellular PK will have the highest priority, followed by mRNA measurements, and then RPPA analyses.
- See Table 9.1.4 for allocation of cells by collection time point.
- For intracellular PK measurements, without delay, PBMC cell pellets will be extracted using perchloric acid (Appendix B) and extracts will be stored frozen at < -70^{o}C prior to shipping.
- For mRNA and RPPA measurements, the washed PBMC pellets will stored frozen at < -70^{o}C pellets prior to shipping.

Table 9.1.4 Allocation of Cells for PK/PD Analysis

	Time Point	Intracellular PK	Cell number	mRNA analysis	Cell number	RPPA + Immunoblot	Cell number
Day 1	Pretreatment	X	1×10^7	X	1×10^7	X	1.5×10^7
	Last 15 min of infusion	X	1×10^7				
	1 hr post EOI	X	1×10^7				
	3 hr post EOI	X	1×10^7	X	1×10^7	X	1.5×10^7
	6-8 hr post EOI	X	1×10^7				
	12-18 hr post EOI	X	1×10^7				
Day 2	Pre-infusion	X	1×10^7	X	1×10^7	X	1.5×10^7
	Last 15 min of infusion	X	1×10^7				

Day 3	Pre-infusion	X	1×10^7				
	Last 15 min of infusion	X	1×10^7				
Day 4	Pre-infusion	X	1×10^7				
	Last 15 min of infusion	X	1×10^7				
Day 5	Pre-infusion	X	1×10^7	X	1×10^7	X	1.5×10^7
	Last 15 min of infusion	X	1×10^7				

9.1.5 Shipping

All PK/PD specimens will be shipped overnight on dry ice to:

Dr. Varsha Gandhi
Room 3SCRB3.4109
Department of Experimental Therapeutics, Unit 1950
MD Anderson Cancer Center
1881 East Road
Houston, TX 77030
Phone: 713 792 2989

9.1.6 Pharmacokinetic Analytic Method

For analysis of human plasma, urine , and cellular samples, a sensitive and selective LC/MS/MS method has been developed and validated at the core facility at MD Anderson to measure primary and active drugs, 8-chloro-adenosine (8-Cl-Ado) and 8-choloro-adenosine triphosphate (8-Cl-ATP), as well as the inactive metabolites 8-chloro-adenine (8-Cl-Ade) and 8-chloro-inosine (8-Cl-Ino).

9.1.7 Pharmacokinetic Data Analysis Methods

Plasma, cellular, and urinary PK data will be analyzed using both non-compartmental and compartmental methods. Non-compartmental PK parameters (e.g. C_{max}, C_{trough}, $AUC_{0\text{-}t}$, CL/F, mean residence time) for 8-chloro-adenosine and its metabolites will be determined using statistical moment theory according to the rule of linear trapezoids. Compartmental PK analyses of the 8-Chloro-adenosine and metabolite data will be performed in ADAPT II software (USC Biomedical Simulations Resource, Los Angeles CA), and secondary PK parameters (e.g. CL_{sys}, V_d, $t_{1/2}$, $AUC_{0\text{-}\infty}$) will be determined for each individual. Finally, summary statistics of the individual non-compartmental and compartmental PK parameters will be derived, and the population PK of 8-chloro-adenosine will be described using a two-stage approach.

9.1.8 Pharmacodynamic Assay Methods

Measurement of short-lived mRNA transcripts in circulating AML blasts.

Briefly, AML blasts will be washed with cold PBS, pelleted, and frozen at -70^{o}C until transport on dry ice to MD Anderson. Three separate pellets will be needed for mRNA (1x10e7), immunoblot (1x10e7) and RPPA (5x10e6). mRNA transcript levels will be measured using real-time RT-PCR techniques. RNA will be isolated from the cell using Qiagen's Qiashredder and RNAeasy kits using routine methods per the manufacturer's instructions. RNA content will be measured using a NanoDrop2000, then primers and probes for specific transcripts will be used and analyzed on an ABI 7900 (TaqMan) sequence detector.

Measurement of short-lived proteins in circulating AML blasts.

For RPPA analysis, following transport to MD Anderson, the cell pellets will be submitted for the CCSG Functional Proteomics Reverse Phase Protein Array (RPPA) Core where the proteins will be extracted and

analyzed on nitrocellulose slides against an extensive set of antibodies. The protein levels will be measured then normalized for protein loading.

9.2 Exploratory *Ex-Vivo* Molecular Studies

9.2.1 Objectives

- To determine the cytotoxicity of 8-chloro-adenosine toward leukemic progenitor cells in vitro.
- To generate a preliminary pre-treatment RNA/miRNA signature in leukemic progenitor cells, and explore its possible association with in vitro cytotoxicity to 8-chloro-adenosine.
- To explore the possible association between the preliminary RNA/miRNA signature and clinical response to 8-chloro-adenosine.

9.2.2 Bone Marrow and PBMC Collection

When feasible an additional 10 ml of bone marrow will be collected in purple-top (EDTA) tube during pre-study evaluation for correlative studies and at the end of cycle 1 and 2 for future studies. If sufficient bone marrow is not collected, PBMC collected from patients with circulating leukemic blasts (via 26 to 30 ml in purple-top (EDTA) tube) will be obtained for analysis. Samples will be gently inverted to mix the sample and then promptly taken to processing by the biospecimen coordinator designated for this study 626-256-4673 ext. 82157 and send email to rbuettner@coh.org in advance of sampling (ideally at least a day in advance).

9.2.3 Initial sample processing

Bone marrow will be processed by Ficoll gradient separation as described in Section 9.1.4 to isolate mononuclear cells.

Bone marrow or blood MNC (5x 10e6) will be used for transcriptome analyses.

Cell pellets will be prepared and stored from remaining cells for RT-PCR and immunoblot assays.

9.2.4 In Vitro and Transcriptome Methods

Primary AML cells will be co-cultured in cytokine containing medium with 8-chloro-adenosine for 48 -72 hrs. The proliferation status of co-cultured cells will be evaluated by Ki67 and propidium iodide (PI) labeling and Cell cycle fractions will be analyzed by flow cytometry. Cultured cells will also be labeled with carboxyfluorescein succinimidyl ester (CFSE) to evaluate cell division. Alternatively the proliferation status of the cells will be assessed by MTS assay. We will use Annexin-V and Caspase-3 labeling to detect apoptosis. The differentiation status of co-cultured cells will be evaluated by analysis of CD34, CD33, CD15, CD14 and CD235 expression. Colony forming cells (CFC) will be evaluated in methylcellulose progenitor assays.

In this aim, we hope to find genes and signaling pathways that are correlated with leukemia cells' response to 8-chloro-adenosine. RNA will be extracted from leukemic progenitors for transcription profiling. The isolated RNA will be submitted to the COH Integrative Genomics Core for whole transcriptome analysis using Illumina Hiseq 2500 to profile total cellular RNA (RNA-seq). The core will also assist with data processing across the full data workflow using both commercial and open-source software packages including R/Bioconductor, IGV, Bowtie, TopHat, Cufflinks, Novoalign, Transfac, BWA, GATK, Samtools, Gene Set Enrichment Analysis (GSEA), Ingenuity Pathway Analysis and DAVID.

Transcripts affected by 8-chloro-adenosine will be further validated using real time RT-PCR assay

10.0 STUDY CALENDAR

Table 10 describes required procedures. All procedures may increase in frequency if clinically indicated.

Cycle length will be 28 days (+/-1 day), unless (a) there is a hold in treatment at Day 1, at which time the previous cycle will be extended until treatment is ready to resume on Day 1 of the subsequent cycle or (b) Day 1 assessments have yet to be reviewed for administering Day 1 agent, at which time the previous cycle will be extended until assessments are reviewed.

The day count continues despite a hold or delay in agent administration. Should a hold or delay in treatment occur, evaluations required by the protocol will be conducted at the original times during the cycle, even if agent(s) is not administered.

The Start of Cycle criteria are found in Section 6.2; **Day 1 treatments may not begin until the respective results of the Day 1 procedures are reviewed.** Windows for procedures follow in the table footnotes.

Table 10.0 Study Activity Calendar

	Pre-Study[a]	Cycles 1-4 [b,c]					30-day post last dose [g]	Active Follow-up [h]	Survival Follow-up [i]
		Day 1[d]	Day 2-5 [e]	Day 8 [f]	Day 15 [f]	Day 22 [f]			
Informed consent [j]	X								
Medical history [k]	X								
Concurrent meds[l]	X	X		X	X	X	X		
Physical exam	X	X		X	X	X	X		
Vital signs[m]	X	X		X	X	X	X		
ECOG Performance Status[n]	X	X					X		
Adverse Event Evaluation[o]		----------X----------					X		
12 lead EKG	X	X[p]	X [p]						
Urinalysis	X	X					X		
B-HCG serum[q]	X								
CBC with diff + platelet count [r]	X	X	X	X	X	X	X		
Chemistry / metabolic panel [s]	X	X	X	X	X	X	X		
Bone Marrow Biopsy	X[t]					X[t,u]			
Response Assessment[v]						X[v]		X[v]	
Cardiac monitoring (telemetry unit)		X[b]	X[b]						
8-chloro-adenosine Infusion[w]		X	X						
Research blood collection	X[x]	X[y]	X[y]						
Research urine collection[z]		X							
Inclusion/Exclusion Criteria [aa]	X								
Registration [bb]	X								
Other anti-cancer treatment								X [cc]	X [dd]
Survival [ee]								X	X

a. Pre-study procedures to be performed within 14 days of start of treatment.

b. **Participants will be hospitalized during treatment administration (at minimum during the first six days of the cycle**). **Patients will undergo continuous cardiac monitoring in telemetry unit from start of treatment until 24 hours after completion of treatment during Cycles 1-4.**

c. Participants will receive a maximum of 4 study cycles. Participants who experience unacceptable toxicity or disease progression after cycle 2 will forgo additional treatment cycles and proceed to the 30-day-follow-up visit. There will be a +7 day window for start of cycles 2-4.

d. Day 1 assessments (including EKG) to be performed prior to Day 1 drug. Start of cycle criteria (Section 6.2) must be met before administering Day 1 study agent.

e. Study agent will be administered daily for five consecutive days. EKG will be performed and reviewed prior to study drug administration. See section 5.2: 'Treatment Cycle Definition' for additional detail regarding treatment administration timing in the event of a hold.

f. Window for Day 8, 15, and Day 22 assessments is the stated day +/- 2 days. Bone marrow biopsy may be performed between days 22 and 28 after cycle initiation.

g. Window for the 30-day Post Last Dose visit is +/- 2 days. All participants will be followed until resolution or stabilization of any related serious adverse events occurring during treatment or starting within 30 days of last study drug administration.

h. Active follow-up will occur for participants who have completed EOT procedures and yet to demonstrate disease progression. The active follow-up visit will take place every 28 days (+/- 5 days) from the day of the EOT Day 30 visit. Active follow-up will continue until an alternative acute leukemia therapy has commenced or until disease progression or up to 2 years following first study agent administration.

i. Survival follow-up will occur approximately every 3 months (+/- 14 days) from Off-Therapy date for survival status and will occur for up to 2 years following the participant's first study agent administration. Information will be obtained via medical record review, use of the social security registry, or by telephone contact.

j. Documented informed consent to occur prior to any study related procedures that are not otherwise SOC.

k. Medical history to include review treatment history for AML, relevant medical history pertaining to study eligibility, and demographic information.

l. Concurrent medications, supportive care, blood products, or radiation therapy taken or administered during the trial will be documented in the subject's medical record using institutional documentation guidelines. See Section 5.11 for prohibited therapies.

m. Vital signs: Weight, heart rate, blood pressure, respiration rate, temp. Height required only at baseline.

n. See Appendix A for ECOG performance status criteria

o. Adverse events (AEs) experienced by participants will be collected and recorded from signing of informed consent document. AE reporting begins for events that occur after registration. AE recording and reporting will continue until the completion of the Day 30-Post-Last-Dose visit or until resolution or stabilization of any related serious AE occurring before the completion the Day 30-Post-Last-Dose visit, whichever occurs later.

p. 12 lead EKG to be performed and reviewed within 3 hours prior to study drug administration AND within 1 hour (-5/+10 minutes) post end of infusion.

q. B-HCG serum pregnancy test for women of childbearing potential only

r. CBC with differential: erythrocytes (RBC), hemoglobin, hematocrit, platelets, total WBC plus absolute differential counts (neutrophils, lymphocytes, monocytes, eosinophils, basophils).

s. Serum chemistry panel: Sodium, potassium, chloride, carbon dioxide, creatinine, urea nitrogen, calcium, glucose, albumin, total bilirubin, alkaline phosphatase, total protein, ALG/SGPT, AST/SGOT, LDH, magnesium, phosphorus, and uric acid.

t. If feasible, from the pre-study, end of cycle 1 and 2 (weeks 4 and 8) bone marrow biopsies, an additional 10 mL will be collected for correlative studies. 10 mL of marrow cells will be collected into a purple top (EDTA) tube, gently agitated, placed on ice and brought promptly (so that the sample

can be processed within the hour) to the Ralf Buetner in Needleman room 1042. Notify Dr. Buetner (or his representative) by e-mail to rbuetner@coh.org in advance of sampling (ideally at least a day in advance). NOTE: if the 10ml research bone marrow sample is not feasible for the pre-study bone marrow, blood may be collected as described in footnote 'x'.

u. Participant will have a bone marrow biopsy after the first and second cycles only (weeks 4 and 8) and when indicated to confirm response. In cases where there is increase of absolute peripheral blood blast count by 50% day 22 bone marrow evaluation can be skipped. For additional correlative sample details see footnote't' or Section 9.2.2. NOTE: if the 10ml research bone marrow sample is not feasible, blood may be collected as described in footnote 'x'.

v. Response assessment to use Döhner, 2010 criteria[24] detailed in Section 12.2. Response will be assessed at the end of each cycle/just prior to the start of each cycle. While response will be assessed at the end of Cycle 1, the result of the end of Cycle 1 response assessment will not affect whether or not a participant continues onto Cycle 2.

w. See Section 5.5 for 8-chloro-adenosine administration details. Study agent will be administered daily for five consecutive days, unless there is a hold due to toxicity. See Section 5.2 for administration and day count when there is a hold in agent administration.

x. For participants who unable to provide the full 10 ml of research bone marrow sample at pre-study (footnotes 't' and 'u'), 26 to 28 ml of peripheral blood will be collected into EDTA purple top tubes. Tubes will be inverted several times and then immediately placed on ice for transportation to Dr. Ralf Buetner in Needleman room 1042. Notify Dr. Buetner (or his representative) by e-mail to rbuetner@coh.org in advance of sampling (ideally at least a day in advance).

y. For Cycle 1 only, blood will be collected for PK/PD analysis from a separate anatomic site (e.g. opposite arm) from site drug infusion at various time points. See Section 9.1.2 for details. Notify the CICSL (Leslie Smith-Powell or her representative) by e-mail to LSmith-Powell@coh.org in advance of sampling (ideally at least a day in advance).

z. Urine will be collected in 8 hr (+/- 30 min) sequential intervals (e.g. 0-8 hrs, 8-16 hrs, and 16-24 hrs) during the first 24 hours after the first dose of cycle 1. Urine may be maintained at RT, and should be brought to CICSL (Shapiro room 1042). Samples may be delivered to CICSL in a single batch.

aa. Inclusion/exclusion criteria are detailed in Section 3.

bb. See Section 4.3 for slot reservation and registration process. Registration is to occur prior to start of treatment. Documentation providing Investigator's confirmation that all eligibility criteria are met must be available and provided for registration.

cc. Participants will be monitored for anti-cancer treatments during Active Follow-Up. If other anti-cancer treatments (including HCT) are initiated, participants will be removed from Active Follow-Up and proceed to Follow-Up for Survival.

dd. Anti-cancer treatments will be collected until the participant completes the study.

ee. Survival information and reason for death will be collected until the participant completes the study.

11.0 DATA REPORTING/PROTOCOL DEVIATIONS

11.1 Data Reporting

11.1.1 Confidentiality and Storage of Records

Electronic Data Collection will be used for this protocol. The data will be stored in encrypted, password protected, secure computers that meet all HIPAA requirements. When results of this study are reported in medical journals or at meetings, identification of those taking part will not be disclosed. Medical records of subjects will be securely maintained in the strictest confidence, according to current legal requirements. They will be made available for review, as required by the FDA, HHS, or other authorized users such as the NCI, under the guidelines established by the Federal Privacy Act and rules for the protection of human subjects.

11.1.2 Subject Consent Form

At the time of registration, the original signed and dated Informed Consent form, HIPAA research authorization form, and the California Experimental Subject's Bill of Rights (for the medical record) and three copies (for the subject, the research record, and the Coordinating Center) must be available. All Institutional, NCI, Federal, and State of California requirements will be fulfilled.

11.1.3 Data Collection Forms and Submission Schedule

All data will be collected using electronic data collection, stored as indicated in Section 12.1.1, and will be submitted according to the timelines indicated in Table 12.1.3.

Table 11.1.3 Data Submission Schedule

Form	Submission Timeline
Eligibility Checklist	Complete prior to registration
On Study Forms	Within 14 calendar days of registration
Baseline Assessment Forms	Within 14 calendar days of registration
Treatment Forms	Within 10 calendar days of treatment administration
Adverse Event Report Forms	For cycle 1 only, within 7 calendar days of AE assessment/notification; for all other cycles, within 10 calendar days of AE assessment/notification
Response Assessment Forms	Within 10 calendar days of the response assessment
Other Assessment Forms (concomitant medications etc.)	Within 10 calendar days of the assessment
Off Treatment/Off Study Forms	Within 10 calendar days of completing treatment or being taken off study for any reason
Follow up/Survival Forms	Within 14 calendar days of the protocol defined follow up visit date or call

Eligibility Checklist

The Eligibility Checklist must be completed by a protocol nurse or clinical research associate and signed by an authorized investigator prior to registering the subject. See Section 4.3 for the registration procedure.

11.2 Protocol Deviations

11.2.1 Deviation Policy

This protocol will be conducted in accordance with COH's "Clinical Research Protocol Deviation Policy" located at http://www.coh.org/dsmc/Documents/Institutional%20Deviation%20Policy.pdf.

Deviations from the written protocol that could increase patient risk or alter protocol integrity require prior IRB approval of a single subject exception (SSE) request. In addition, if contractually obligated, the sponsor must also approve the deviation. IRB pre-approved SSE protocol modifications are considered an amendment to the protocol and not a deviation. The submission of a deviation report is not required.

Brief interruptions and delays may occasionally be required due to travel delays, airport closure, inclement weather, family responsibilities, security alerts, government holidays, etc. This can also extend to complications of disease or unrelated medical illnesses not related to disease progression. The PI has the discretion to deviate from the protocol when necessary so long as such deviation does not threaten patient safety or protocol scientific integrity. Examples include, but are not limited to: a) dose adjustments based on excessive patient weight; b) alteration in treatment schedule due to non-availability of the research participant for treatment; c) laboratory test results which are slightly outside the protocol requirements but at levels that do not affect participant safety. These instances are considered to be deviations from the protocol. A deviation report will be submitted to the DSMC/IRB within five days.

11.2.2 Reporting of Deviations

All deviations will be reported to the COH DSMC within five days. The DSMC will forward to report to the IRB following review.

11.2.3 Resolving Disputes

The COH Investigational Drug Service (IDS) cannot release a research agent that would cause a protocol deviation without approval by the PI. Whenever the protocol is ambiguous on a key point, the IDS should rely on the PI to clarify the issue.

In situations where there is misperception or dispute regarding a protocol deviation among the persons involved in implementing the protocol, it is the responsibility of the PI to resolve the dispute and the PI may consult with the DSMC chair (or designee) to arrive at resolution.

12.0 ENDPOINT EVALUATION CRITERIA/MEASUREMENT OF EFFECT

12.1 Toxicity

The primary endpoint is toxicity. Toxicity will be graded according to the NCI-Common Terminology Criteria for Adverse Events version 4.03. Dose limiting toxicity (DLT) is defined in section 13.2 of the protocol. The MTD will be based on the assessment of DLT during cycle 1.

12.2 Response

The primary endpoint is complete remission rate (CR + CRi) and is based on the Döhner, 2010 criteria[24]; calculated as the percent of evaluable patients that have confirmed CR or CRi.

Response criteria will be as follows:

CR: Bone marrow with $< 5\%$ blasts by morphology. ANC $> 10^3/\mu L$, platelets $\geq 10^5/\ \mu L$
CRi: Bone marrow with $< 5\%$ blasts by morphology, not meeting blood count criteria for CR.

PR: Decrease of at 50% in bone marrow blasts
PD: Bone marrow blasts more than 20% and increase in blasts by over 50%.
HR: improvement in hematologic parameters, but not meeting criteria for PR.

Secondary activity endpoints for this study are as follows:

- Duration of response, defined as the time interval from the date of first documented response (CR+CRi) to documented disease relapse or death whichever occurs first.
- Overall survival, defined as the time interval from date of first dose of study drug to date of death from any cause.
- Event-free survival, defined as the time interval from date of first dose of study drug to first documented disease progression or death from any cause, whichever occurs first.
- Overall response rate (CR/CRi/PR), calculated as the percent of evaluable patients that have confirmed CR or CRi or PR.

12.3 Clinical Pharmacology Endpoints:

- For plasma PK, levels of 8-chloro-adenosine, its deaminated metabolite, 8-chloro-inosine, and its base 8-chloro-adenine will be quantitated in plasma during 5 days of therapy.
- For cellular PK, concentrations of 8-chloro-adenosine and 8-chloro-ATP will be quantitated in circulating leukemia cells in the peripheral blood.
- For urine PK, levels of 8-chloro-adenosine, its deaminated metabolite, 8-chloro-inosine, and its base 8-chloro-adenine will be quantitated in urine during the first 24 hours of therapy.
- For PD, the impact of therapy on endogenous ATP pools, as well as the effect on short-lived mRNA transcripts and proteins will be analyzed in circulating acute leukemia blasts.

13.0 STATISTICAL CONSIDERATIONS

13.1 Study Design

This study will be conducted as a single center, single agent, phase I/II trial of 8-chloro-adenosine. 8-chloro-adenosine will be administered as a 4 hour infusion daily for 5 days every 4 weeks; treatment cycle length is 28 days.

Phase I:

The primary objective of the phase I study is to determine the recommended phase II dose (RP2D) of 8-chloro-adenosine when given as a single agent, in patients with relapsed or refractory acute myeloid or lymphoblastic leukemia. The dose of 8-chloro-adenosine will depend on dose level assignment.

The phase I study will follow a modified 3+3 design for enrollment with dose escalation, or expansion of a cohort on the basis of the occurrence of dose limiting toxicities (DLTs) during cycle 1. This modified design involves an initial accelerated dose escalation stage (dose-doubling from an initial dose of 100mg/m^2 to 800mg/m^2) that may be ended by a single DLT in cycle 1 or two occurrences of 'moderate' treatment-related toxicity. During the initial accelerated stage of accrual, cohorts of up to 3 patients will be enrolled and the dose level will be escalated by increments of 100% over four doses of 8-chloro-adenosine (dose level 1a: 100mg/m^2; dose level 2a: 200 mg/m^2; dose level 3a: 400 mg/m^2 and dose level 4a: 800 mg/m^2). If the accelerated dose stage is halted due to toxicity, the study will switch to escalation that includes intermediate doses. The intermediate dose levels –'b' levels, in increments of 50% from the planned four doses, are defined as follows: 150mg/m^2; 300 mg/m^2; and 600 mg/m^2. **With Amendment v07**: the starting dose will be 400 mg/m^2 (dose level 3a).

At the conclusion of the accelerated stage, dose escalation will occur according to the standard 3+3 rules: The highest dose level that produces ≤1/6 DLTs in cycle 1 will be the maximum tolerated dose (MTD). The RP2D of 8-chloro-adenosine will generally be the MTD, but it may be less than the MTD based on a review of available data/cumulative toxicities from phase I. The RP2D identified in the phase I portion of the study will be brought forward for activity evaluation.

Phase I (Evaluable for Toxicity): Patients will be considered evaluable for toxicity if they receive any study drug. To be evaluable in the context of dose escalation, a patient must receive at least 80% of 8-chloro-adenosine and be followed for the full 28 days during cycle 1 or experience a DLT during the first cycle of therapy. All patients who are not evaluable for DLT will be replaced.

Phase II:

The primary objective is to estimate the response rate and to evaluate the antitumor activity of 8-chloro-adenosine, in patients with relapsed or refractory acute myeloid or lymphoblastic leukemia. The primary endpoint is complete remission rate (CR/CRi) and is based on the Döhner, 2010 criteria[24]. A single cycle of treatment will be given in a 28 day cycle. Each patient's disease status will be evaluated at baseline. Response will be assessed at the end of each cycle/just prior to the start of each cycle.

The phase II portion of this study will implement a Gehan two-stage design to estimate the response rate and to evaluate the activity of 8-chloro-adenosine when given as a single agent [23]. The phase II portion of the study is expected to enroll a minimum of 9 and a maximum of 25 patients. The six patients treated at the RP2D in the phase I portion of the study will count toward the 25 patients required; given this, we expect to enroll only 19 new patients on the phase II trial. The sample size is based on the desire to estimate the response rate with at most 10% standard error, and early stopping if the treatment is unexpectedly ineffective.

At stage 1, 9 patients will be entered on the study. If 0 responses are seen in the first 9 patients treated, the study will be terminated and the true regimen response will be declared ≤30%. If at least 1 patient responds, the trial will continue to the second stage. Because patients treated during the phase I portion of the trial at the dose selected for the phase II trial will be counted (n=6), only 3 additional patients will be enrolled at stage 1. Under this design if the study agent is >30% effective, there would be 96% chance of at least one success.

At stage 2, 16 additional patients will be entered. This accrual provides for estimation of the response rate with no more than 10% standard error.

Phase II (Evaluable for Toxicity): Patients will be considered evaluable for toxicity if they receive any study drug. Patients enrolled/treated as part of the phase II study will not be replaced based on toxicity.

Phase I and II (Evaluable for Response): For the phase II portion, as part of the primary analysis, patients will be considered evaluable for response if they are eligible, have baseline disease assessments, and receive 2 cycles of protocol treatment or achieve a CR/CRi after 1 cycle of protocol treatment. Patients will have their response classified according to the Dohner 2010 criteria[24].

13.2 Definition of Dose-Limiting Toxicity (DLT)/Moderate Toxicity

With Amendment v09: A two level toxicity attribution scale will be used. In terms of attribution, all recorded adverse events will be classified as either related or unrelated to the study drug. Prior to this amendment a five level attribution scale (definite, possible, probable, unlikely, unrelated) was used.

A DLT will be defined as any of the following toxicities that occur during cycle 1, per CTCAE version 4.03, and are considered related to the study drug:

(Note: All adverse events will be considered relevant to determining dose-limiting toxicities and reporting unless the event can clearly be determined to be unrelated to the drug.)

Hematologic toxicities:

- For participants with less than 5% bone marrow blasts by morphology, with the exception of Grade 4 neutropenia, Grade 3 or 4 hematologic toxicity that does not resolve to Grade 2 by Day 49 (21 days after the day 28 cycle).
- For participants with less than 5% bone marrow blasts by morphology, Grade 4 neutropenia (absolute neutrophil count $<0.5 \times 10^9$/L) persisting up to Day 42 after the start of treatment.

Non-Hematologic toxicities:

- Any grade ≥3 non-hematologic toxicity for ≥7 days, with the exception of nausea not controlled by medical management within 72 hours.
- Any treatment related toxicity that prevents the administration of 80% of 8-chloro-adenosine during the first cycle of therapy.
- Delay in start of cycle 2 by more than 14 days due to drug-related non-hematologic toxicity.

Moderate toxicity will be defined as any grade 2 non-hematologic toxicity and occurs during cycle 1, per CTCAE v4.03, with the exception of:

- Allergic rhinitis
- Fatigue
- Sweating
- Weight gain or loss
- Alopecia
- Dry skin
- Nail or pigmentation changes
- Pruritus
- Hot flashes
- Flatulence
- Mouth dryness
- Nausea and vomiting without the use of maximal anti-emetic treatment
- Sense of smell or taste disturbance
- Erectile impotence
- Irregular menses
- Libido
- Oligospermia
- Tumor lysis syndrome
- Electrolyte/metabolic toxicity unable to be corrected within 48 hours
- Other grade 2 toxicity may be excluded pending PI and Data Safety Monitoring Committee (DSMC) review and approval

(Note: All adverse events will be considered relevant to determining moderate toxicities and reporting unless the event can clearly be determined to be unrelated to the drug.)

13.3 Dose Escalation/Expansion

The phase I study will follow a modified 3+3 design. During dose-doubling, enrollment and dose escalation of a cohort will be on the basis of the occurrence of moderate and/or dose limiting toxicities (DLTs). If dose-doubling is ended by a single DLT in cycle 1 or two occurrences of 'moderate' treatment-related

toxicity, subsequent dosing will be based on DLT only. DLT and moderate toxicity incidence will be based on toxicity events encountered during the first cycle of 8-chloro-adenosine treatment.

This modified design involves an initial accelerated dose escalation stage (dose-doubling over 'a' levels) that may be ended by a single dose-limiting toxicity (DLT) in cycle 1 or two occurrences of 'moderate' treatment-related toxicity as defined in section 13.2. During the initial accelerated stage of accrual, cohorts of up to 3 patients will be enrolled and the dose level will be escalated by increments of 100% (skipping 'b' dose levels). If two patients encounter moderate toxicity or one patient encounters DLT during cycle 1, the current dose level will be expanded and the accelerated stage will end; dose-doubling will stop. At the conclusion of the accelerated stage, dose escalation will occur over 'a' and 'b' dose levels according to the following rules*:

- If zero out of 3 evaluable patients has a DLT in cycle 1 then the next dose of 8-chloro-adenosine will be tested.
- If 1 out of 3 evaluable patients has a DLT in cycle 1, three additional patients will be assessed at the same dose of 8-chloro-adenosine.
- If 2 out of 3 patients have a DLT in cycle 1, dose escalation will cease and the next lower dose level will be expanded. Note: If 2 of 3 experience DLT on dose level -1, the trial will be stopped. Dose levels below 50mg/m^2 will not be considered.
- If 1 out of 6 evaluable patients has a DLT in cycle 1, then dose escalation to the next dose of 8-chloro-adenosine will continue.
- If 2 or more out of 6 patients have a DLT in cycle 1, dose escalation will cease and the next lower dose level will be expanded. Note: If 2 of 6 experience DLT on dose level -1, the trial will be stopped. Dose levels below 50mg/m^2 will not be considered.

The highest dose level that produces ≤1/6 DLTs in cycle 1 will be the maximum tolerated dose (MTD). Patients may continue therapy unless there is unacceptable toxicity, disease relapse/progression or withdrawal of consent.

*: Note: Dose de-escalation, escalation or cohort expansion will only take place after 3 patients are fully assessed (assuming the first two patients have yet to experience a DLT) using the Common Terminology Criteria for Adverse Events (CTCAE) of the National Cancer Institute (NCI) version 4.03 following the completion of cycle 1.

13.4 Recommended Phase II Dose (RP2D)

The MTD will be based on the assessment of DLT during cycle 1 (see section 13.2). The MTD will be defined as the highest dose at which ≤ 1/6 patients in a cohort experience DLT. The recommended phase II dose (RP2D) of 8-chloro-adenosine will generally be the MTD, but it may be less than the MTD based on a review of available data/cumulative toxicities from phase I.

13.5 Sample Size Accrual Rate

Phase I:

The phase I study will follow a 3+3 design, to evaluate toxicities associated with 8-chloro-adenosine when given as a single agent. Four doses of 8-chloro-adenosine will be tested: dose level 1a: 100 mg/m^2; dose level 2a: 200 mg/m^2; dose level 3a: 400 mg/m^2 and dose level 4a: 800 mg/m^2. Intermediate dose levels, in increments of 50% from the planned four doses, will be explored in the presence of dose limiting toxicity or unacceptable moderate toxicity. Intermediate doses –'b' levels are defined as follows: 150mg/m^2; 300 mg/m^2; and 600 mg/m^2. In the phase I portion of this study, the total sample size will depend on the

number of dose levels evaluated to determine the RP2D. While the phase I study is expected to enroll and treat 15 patients (dose levels 1-3 –'a' levels, to enroll/treat 3 patients each for a total of 9, and another 6 at dose level 4-assuming the 800 mg/m^2 dose is well tolerated), a maximum of 24 patients could be treated (6 patients treated at each of the four dose levels) –assuming intermediate doses are not tested.

Phase II:

The phase II portion of the study is expected to enroll a minimum of 9 and a maximum of 25 patients. The six patients treated at the RP2D in the phase I portion of the study will count toward the 25 patients required; given this, we expect to enroll only 19 new patients during the phase II trial portion. The sample size is based on the desire to achieve a (promising) target response rate of >30%.

Clinical Pharmacology (PK/PD):

Pharmacokinetic (PK) will be performed during Cycle 1 in all enrolled patients in Phase 1 and Phase 2 portions of this protocol. In addition, pharmacodynamics measurements will be performed during Cycle 1 in all enrolled patients in the Phase 1 and 2 portions who have $\geq$5000 circulating leukemic blasts per microliter.

Molecular Studies:

For mRNA-seq, sequence reads will be mapped to the human genome (hg19) using TopHat and the frequency of Refseq genes will be counted with customized R scripts. For miRNA-seq, sequences will be adapter trimmed and aligned to hg19 genome using Novoalign, and miRNA expression level will be counted as previously described [1]. The raw counts will then be normalized and compared using the Bioconductor package "edgeR" [2]. Fold changes > than 3 with a false discovery rate (FDR) < 0.05 will be considered significant. Assuming the dispersion of genes across replicates is 0.2, 12 samples in each group (responder vs. non-responder) should have 88% power to detect a 3-fold difference between groups at an FDR level of 0.05.

13.6 Safety Analysis and Stopping Rules for Excessive Toxicity

Following the phase I study, the early stopping rule for safety/toxicity will be assessed for each patient after cycle 1. The expected rate of unacceptable toxicity should not be ≥33%. Note: The phase II portion of the study will use the DLT definition to define unacceptable toxicity (see section 13.2). This rule is in addition to the quarterly review of all toxicities submitted to the COH DSMC. Given the number of patients treated, if the unacceptable toxicity rate is ≥33%, patient accrual will be halted and a full review of the data by the Data Safety Monitoring Committee (DSMC) will be mandated.

In addition, cumulative safety data will be reviewed on a quarterly basis in order to identify safety concerns that may emerge due to cumulative exposure.

13.7 Trial Statistical Analysis Plan

Patient demographic and baseline characteristics, including age, gender, medical history, and prior therapy, will be summarized using descriptive statistics. For continuous variables, descriptive statistics (number [n], mean, standard deviation, standard error, median (range) will be provided. For categorical variables, patient counts and percentages will be provided.

Phase I Analysis: Observed toxicities will be summarized in terms of type (organ affected or laboratory determination), severity, time of onset, duration, probable association with the study treatment and reversibility or outcome. Baseline information (e.g. the extent/type of prior therapy) and demographic information will be presented as well to describe the patients treated in this study.

Phase II Analysis: The complete remission (CR+CRi) rate will be calculated as the percent of evaluable patients; exact 95% confidence intervals will be calculated for these estimates. Response rates will also be evaluated based on number and type of prior therapy(ies). Time to response, duration of response, and survival will be estimated using the product-limit method of Kaplan and Meier.

Clinical Pharmacology Analysis Plan:

- Plasma, urine and cellular PK of 8-chloro-adenosine and metabolites will be quantitated as continual measurement variables as per assays described in Section 9.1.7.
- Descriptive statistics and graphical displays will be used to summarize levels of serum/urine 8-chloro-adenosine and its metabolites at each time point and to evaluate changes pre- post-treatment measurement. A paired t-test will be used to determine if there is a statistically significant change.
- Similar methods will be used to assess the PD impact of 8-chloro-adenosine on the cellular ATP pool and select short-lived mRNAs including corresponding proteins in AML blasts. Protein and mRNA levels will be summarized descriptively using means, medians, standard deviations and ranges.
- Summary statistics of the PK/PD parameters for the population will be derived from the parameters obtained from the individual patients.
- Results will also be summarized in terms of response and toxicity.
- To determine if there are dose-dependent changes in 8-chloro-ATP and 8-chloro-adenosine (when administered ex vivo), summary statistics and graphical displays will be generated to descriptively compare accumulation of 8-chloro-ATP and endogenous ATP levels in circulating AML blasts over 5 doses.
- Gene ontology (GO) and pathways will be analyzed using DAVID (Database for Annotation, Visualization, and Integrated Discovery), an online bioinformatic resource for functional interpretation of large lists of genes. Pathways and GO that correlate with response will be sorted by their multiple comparison adjusted p value. Potential miRNA targets will be identified using TargetScan and their functional annotation will be done using DAVID.
- All summaries will be exploratory in nature, with the goal of developing further questions regarding the modulation of therapy, or regarding reasons for efficacy or lack of efficacy.

14.0 HUMAN SUBJECT ISSUES

14.1 Institutional Review Board

In accordance with City of Hope policies, an Institutional Review Board (IRB) that complies with the federal regulations at 45 CFR 46 and 21 CFR 50, 56 and State of California Health and Safety code, Title 17, must review and approve this protocol and the informed consent form prior to initiation of the study. All institutional, NCI, Federal, and State of California regulations must be fulfilled.

14.2 Recruitment of Subjects

Participants will be recruited from patients undergoing treatment for acute myeloid leukemia at City of Hope Cancer Center for Hematological Malignancies.

Any advertisements will be reviewed and approved by the IRB prior to their use to recruit potential study subjects.

14.3 Study location and Performance Sites

This study will be performed at COH.

14.4 Confidentiality

This research will be conducted in compliance with federal and state of California requirements relating to protected health information (PHI). The study will record individual side effects to study treatment, and disease status, and these will be linked to the subject's identity using a coded study number. The principal investigator, co-investigators, and laboratory technicians will have access to this information, but all information will be treated confidentially. No identifiers will be used in any subsequent publication of these results.

14.5 Financial Obligations and Compensation

The investigational agent, 8-chloro-adenosine, will be provided free of charge by study for the duration of the trial.

The standard of care drug(s) and procedures provided will be the responsibility of the research participant and/or the insurance carrier. The research participant will be responsible for all copayments, deductibles, and other costs of treatment and diagnostic procedures as set forth by the insurance carrier. The research participant and/or the insurance carrier will be billed for the costs of treatment and diagnostic procedures in the same way as if the research participant were not in a research study. However, neither the research participant nor the insurance carrier will be responsible for the research procedures related to this study.

In the event of physical injury to a research participant, resulting from research procedures, appropriate medical treatment will be available at the City of Hope to the injured research participant, however, financial compensation will not be available.

The research participant will not be paid for taking part in this study.

14.6 Informed Consent Processes

The Principal Investigator or IRB approved named designate will explain the nature, duration, purpose of the study, potential risks, alternatives and potential benefits, and all other information contained in the informed consent document. In addition, they will review the experimental subject's bill of rights and the HIPAA research authorization form. Research subjects will be informed that they may withdraw from the study at any time and for any reason without prejudice, including as applicable, their current or future care or employment at City of Hope or any relationship they have with City of Hope. Research subjects will be afforded sufficient time to consider whether or not to participate in the research.

Prospective research subjects who cannot adequately comprehend the fundamental aspects of the research study with a reasonable amount of discussion, education and proctoring will be ineligible for enrollment. For those subjects who do comprehend the fundamental aspects of the study, consent will be obtained and documented, followed by eligibility testing. The research team will review the results of eligibility testing and determine if the subject is a candidate for study enrollment.

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APPENDIX A: ECOG PERFORMANCE STATUS CRITERIA

ECOG Performance Status Scale	
Grade	**Criteria**
0	Normal activity. Fully active, able to carry on all pre-disease performance without restriction.
1	Symptoms, but ambulatory. Restricted in physically strenuous activity, but ambulatory and able to carry out work of a light or sedentary nature (e.g., light housework, office work).
2	In bed <50% of the time. Ambulatory and capable of all self-care, but unable to carry out any work activities. Up and about more than 50% of waking hours.
3	In bed >50% of the time. Capable of only limited self-care, confined to bed or chair more than 50% of waking hours.
4	100% bedridden. Completely disabled. Cannot carry on any self-care. Totally confined to bed or chair.
5	Dead.

APPENDIX B: PERCHLORIC ACID EXTRACTION FOR TRIPHOSPHATE POOL ANALYSIS

Provided by Dr. Varsha Ghandi of MD Anderson Cancer Center

Keep cold during entire procedure

1. Count cells and harvest 5×10^6 cells.
2. Centrifuge cells (4°C, 1500rpm, 5mins)
3. Remove supernatant
4. Wash with cold PBS
5. Centrifuge cells (4°C, 1500rpm, 5mins)
6. Remove supernatant
7. Repeat steps 4-6
8. Add 250 μL cold Milli-Q water and 250 μL 0.8 N PCA
9. Incubate on ice for 5 mins
10. Centrifuge cells (4°C, 1500rpm, 5mins)
11. Transfer supernatant to a new 2.0mL tube
12. To pellet, add 250 μL 0.4 N PCA
13. Incubate on ice for 5 mins
14. Centrifuge cells (4°C, 1500rpm, 5mins)
15. Transfer supernatant to 2.0mL tube from step 11
16. Add 30 μL 10 N KOH
17. Continue to neutralize with 1 N KOH and 3 N KOH and 0.8 N PCA
18. Centrifuge cells (4°C, 1500rpm, 5mins)
19. Transfer supernatant to a new 1.7mL tube and add milli-Q water to a final volume of 1 mL using a glass Hamilton syringe. Do not get any of the pellet or salt into the sample.
20. Store at −20°C until HPLC analysis.