COLUMBIA UNIVERSITY MEDICAL CENTER

TITLE: **PHASE I/IIA STUDY OF THE ORAL 5-AZACITIDINE IN COMBINATION WITH THE HISTONE DEACETYLASE INHIBITOR ROMIDEPSIN FOR THE TREATMENT OF PATIENTS WITH T-CELL LYMPHOMA AND GERMINAL-CENTER-DERIVED B-CELL LYMPHOMA**

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CUMC Protocol #: AAAM3752

Protocol Type / Version # / Version Date: *Amendment 1.4/ Version 1 / 20June2018*

ClinicalTrials.gov Identifier: NCT01998035

PROTOCOL SYNOPSIS

Title:

Phase I/IIa study of oral 5-azacitidine in combination with the histone deacetylase inhibitor romidepsin for the treatment of patients with T-cell lymphomas

Study Design:

This is an open label, phase I/IIa, 3 x 3 dose-escalation study with an initial phase I followed by a disease focused phase II.

The primary objective of the phase I is to determine the maximum tolerated dose (MTD) and dose limiting toxicity (DLT) of the combinations of oral 5-azacitidine and romidepsin in patients with lymphoma. The safety and toxicity of this combination will be evaluated throughout the entire study.

If the combination of oral 5-azacitidine and romidepsin is found to be feasible and an MTD is established, the phase II part of the study will be initiated.

Phase II will consist of a 2 stage design of the combination of oral 5-Azacitidine and romidepsin for patients with relapsed or refractory T-cell lymphomas.

Objectives:

A. Phase I:

Primary Objectives

- Determine the maximum tolerated dose (MTD) and dose limiting toxicity (DLT) of the combination of oral 5-azacitidine and romidepsin.
- Evaluate the safety and toxicity of the combination of oral 5-azacitidine and romidepsin.

Secondary Objectives

- Describe the maximum number of cycles received
- Describe the number of dose delays and dose reductions at the MTD
- Describe the anti-tumor activity of the combination
- Evaluate the overall response rate (ORR), progression free survival (PFS), and duration of response (DOR) of the study population.

Exploratory Objectives

- Evaluate pharmacodynamic markers of drug effect in paired tissue biopsies (pre- and post- treatment) including gene expression and genome wide methylation patterns.
- Establish the pharmacokinetic profile for oral 5-azacitidine and romidepsin when given as a combination in Cycle 1 at various time intervals as listed in Figure 1.

B. Phase II:

Primary Objectives

- Estimate the ORR (complete + partial response) of the combination of oral 5-azacitidine and romidepsin in patients with relapsed/refractory T-cell Lymphoma.

Secondary Objectives

- Estimate the DOR and PFS of the combination in patients with T-cell lymphoma.
- Estimate the overall survival of patients with T-cell lymphoma on study.
- Identify potential pre-treatment biomarkers of response based on GEP and/or methylation array) to clinical outcome.

Exploratory Objectives

- Evaluate pharmacodynamic markers of drug effect in paired tissue biopsies (pre- and post- treatment) including gene expression and genome wide methylation patterns.

C. Exploratory Cohort: Germinal Center B-cell Lymphoma:

Primary Objectives

- Determine the overall response rates (complete responses + partial responses) of oral azacytidine and romidepsin in patients with GC-derived B-cell lymphomas.
- Determine Progression Free Survival (PFS) and Time to Treatment Failure (TTF)
- Determine the Duration of Response (DOR)

Secondary Objectives:

- Determine the safety and tolerability of oral azacytadine and romidepsin in patients with relapsed or refractory GC-derived B-cell lymphoid malignancies.
- Evaluate the pharmacokinetic (PK) profile of oral azacytadine and romidepsin.
- Evaluate and compare pharmacodynamic (PD) endpoints with PK endpoints. PD endpoints will include the evaluation of paired gene expression profiles of primary patient samples on study.

Target Population

Phase I: Patients with relapsed or refractory non-Hodgkin lymphoma, Hodgkin lymphoma
Phase II: Patients with untreated or relapsed or refractory T-Cell Lymphoma
Exploratory Cohort: Patients with relapsed or refractory Germinal Center-Derived B-Cell Lymphoma

Inclusion Criteria

- **Phase I:** Patients must have histologically confirmed relapsed or refractory non-

Hodgkin lymphoma or Hodgkin lymphoma (defined by WHO criteria), with no accepted curative options.
Phase II: Patients must have histologically confirmed T-cell lymphoma (as defined by WHO criteria), including central nervous system (CNS) involvement or lymphomatous meningitis.
Exploratory Cohort: Patients with histologically confirmed relapsed or refractory Germinal Center (GC)-derived B-Cell Lymphoma (diffuse large B-cell (DLBCL) and follicular lymphoma (FL)) defined by the WHO and Hans criteria with no accepted curative options. (Appendix 5)

- There is no upper limit for the number of prior therapies
- Evaluable Disease in the Phase I, and measurable disease as defined in Section 11.1 in the Phase II.
- Age ≥18 years.
- ECOG performance status ≤2
- Patients must have adequate organ and marrow function as defined in Section 3.1.6
- Adequate Contraception
- Ability to understand and the willingness to sign a written informed consent document.

Exclusion Criteria

- Prior Therapy
    - Exposure to chemotherapy or radiotherapy within 2 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 2 weeks earlier.
    - Systemic steroids that have not been stabilized (≥ 5 days) to the equivalent of ≤10 mg/day prednisone prior to the start of the study drugs.
    - No other investigational agents are allowed.
- History of allergic reactions to oral 5-azacitidine or romidepsin.
- Uncontrolled inter-current illness
- Pregnant women
- Nursing women
- Current malignancy or history of a prior malignancy, as outlined in Section 3.3.7
- Patient known to be Human Immunodeficiency Virus (HIV)-positive
- Active Hepatitis A, Hepatitis B, or Hepatitis C infection

Treatment Plan (Phase I)

Patients will be treated with oral 5-azacitidine and romidepsin administered as follows (detailed in Tabel 2): escalating oral 5-azacitidine from Days 1-14 (Dose cohorts -1 to 5)or Days 1-21 (Dose Cohort 6); and romidepsin administered intravenously on Days 8, 15 (Dose cohorts 1-4) of a 28 day cycle and Day 22 (Dose Cohorts 5 and 6) of a 35 day cycle. Cohorts of 3 patients will be enrolled sequentially as outlined in the dose escalation scheme

(Table 2). Once the MTD is reached the Phase II part of the protocol will be initiated in patients with T-Cell Lymphoma (Figure 2).

Treatment Plan (Phase II and Exploratory Cohort)

Patients will be treated with oral 5-azacytidine and romidepsin at the MTD (i.e the Recommended Phase 2 Dose, or RP2D). The treatment will be administered as follows: oral 5-azacytidine 300 mg (flat dose) Days 1-14 and romidepsin 14 mg/m2 on Days 8, 15, and 22 on an every 35 day cycle. .

Duration of Treatment

Patients will be treated until one of the following events:

- Disease progression
- Unacceptable adverse event(s), DLTs
- Withdrawal of consent
- General or specific changes in the patient's condition that render the patient unacceptable for further treatment in the judgment of the investigator
- An event that in the judgment of the treating physician warrant's discontinuation of therapy.

Sample Size

Phase I

An estimated 36 patients (max of 45) will be accrued to the initial Phase I study; a minimum of 3 patients will be treated at each of the 6 dose levels (Dose Escalation and de-escalation; Tables 3 & 4). If a DLT is reached early we will treat fewer patients. We will expand the cohort which meets criteria for a DLT to a total of 6.

Phase II

The phase II part of the study will accrue a total of 24 patients.

Exploratory Cohort

The exploratory cohort part of the study will accrue a total of 10 patients.

Safety

Patients will be monitored carefully for the development of adverse events as well as clinical and/or radiographic evidence of disease response. Adverse events will be evaluated according to criteria outlined in the NCI Common Terminology Criteria for Adverse Events (CTCAE), version 4.0.

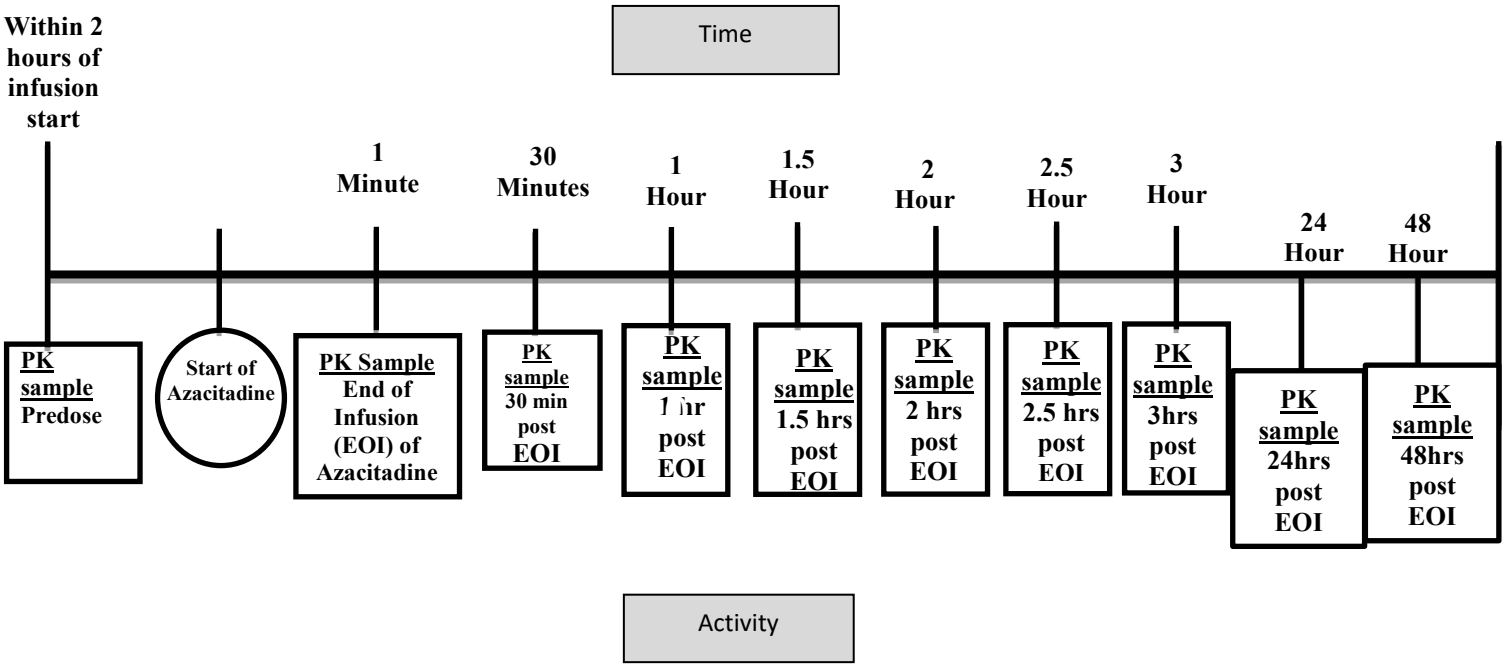
Efficacy Outcome

Overall response rates will be evaluated using clinical parameters, CT scan (PET/CT scan is optional) and bone marrow biopsy, as outlined by the 2007 International Harmonization Project criteria.[1]

Pharmacokinetic Analyses (T-Cell Population ONLY)

- Pharmacokinetic studies will be performed on up to 10 patients in the Phase 2. A pre-treatment blood sample will be obtained prior to starting 5-azacytidine and romidepsin. On day 8 of cycle 1, blood samples will be obtained from patient's at fixed time intervals after administration of Romidepsin ($t_{1/2}$ = 3.5 hours) to determine the plasma level of both romidepsin and oral 5-azacitidine. The patient will be directed to take 5-azacytidine at the time the romidepsin infusion begins. This will be compared to the known pharmacokinetic data for the individual agents in order to identify any possible effect of the combination. The proposed time points are shown in Figure 1.

Figure 1: Drug Administration and Pharmacokinetic Sampling Schedule (Day 1)



* All PKs should be drawn within 15 minutes of specified time.

Figure 1a: Drug Administration and Pharmacokinetic Sampling Schedule (Day 8)

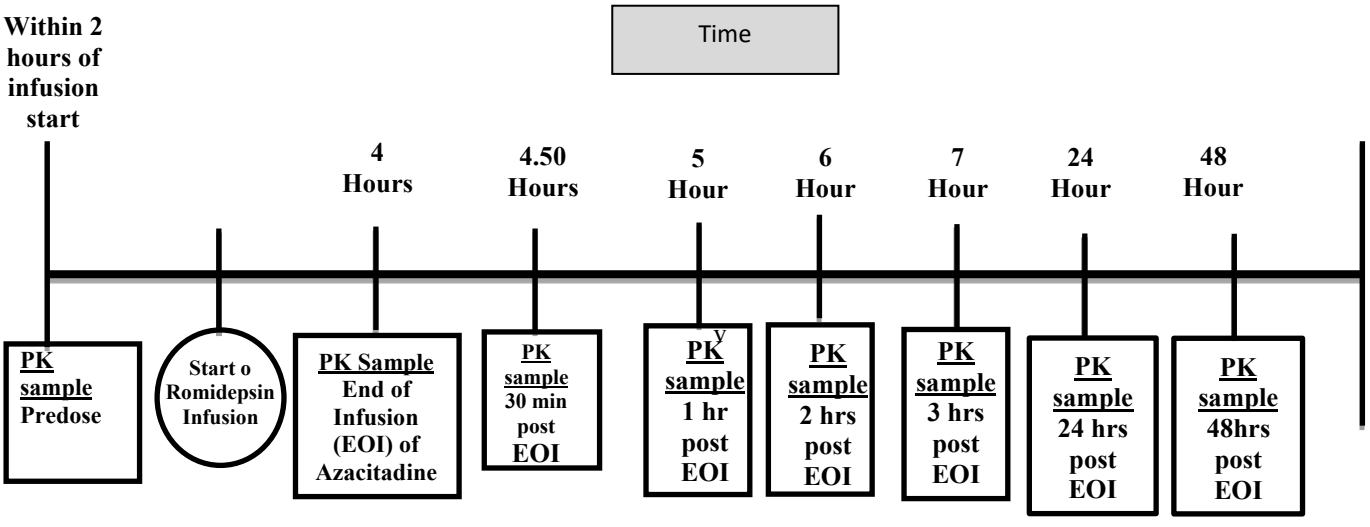
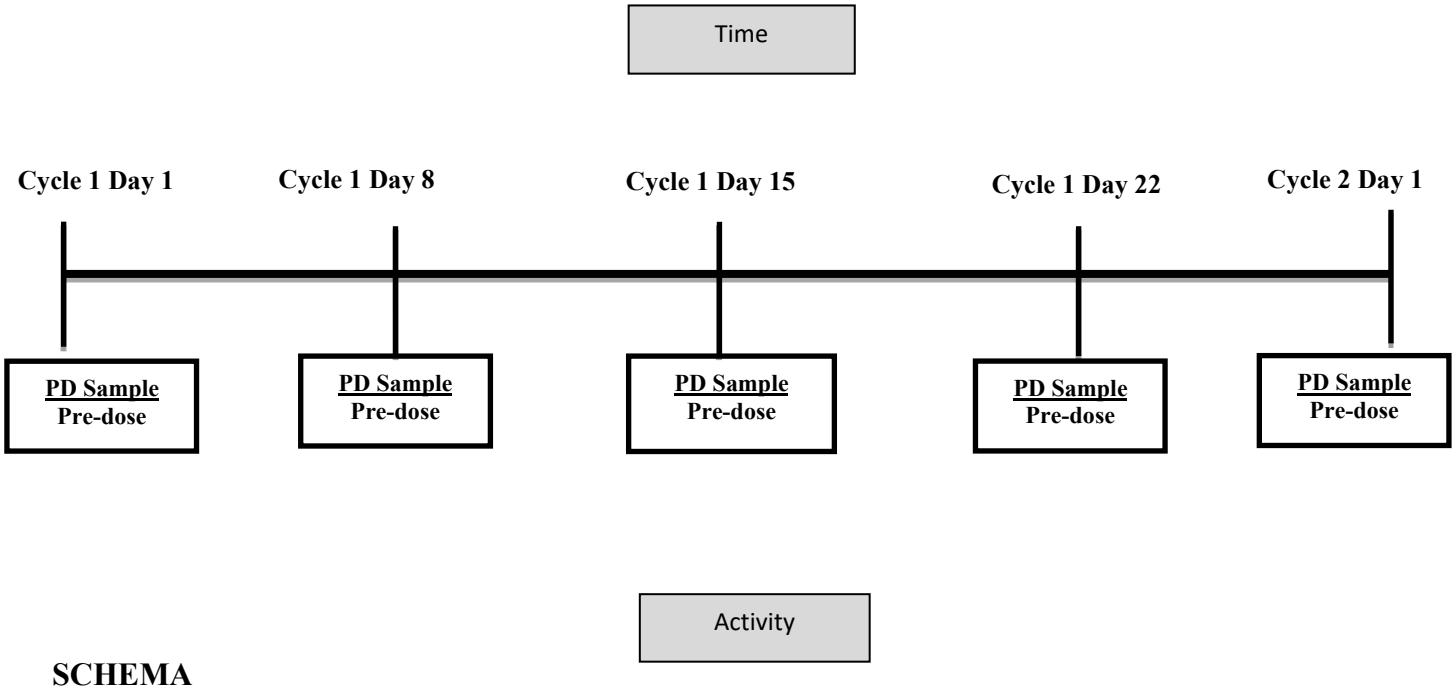


Figure 1b: Drug Administration and Pharmacodynamic Sampling Schedule



SCHEMA

Table 1: Regimen Description

REGIMEN DESCRIPTION					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Oral 5-azacitidine	Standard Premedications	300 mg (flat dose)	PO Days 1-14	Days 1-14	*35 days*
Romidepsin	Antiemetic	14 mg/m2	IV over 4 hours	Days 8, 15 and 22	
** *Doses as appropriate for assigned dose level.*					

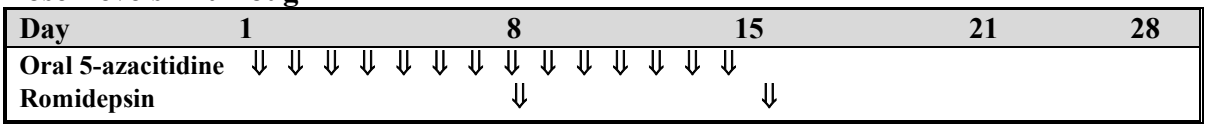
Table 2: Dose Escalation Scheme for the Combination of Oral 5-azacitidine and Romidepsin

Dose Escalation Schedule			
Dose Level	Dose		
	Oral 5-azacitidine (Flat dose [mg])	*Romidepsin (mg/m2 rounded to 14mg/m2)*	*Cycle Length*
Level -1	100 (Days 1-14)	10 (Day 8)	28
Level 1	100 (Days 1-14)	10 (Days 8 & 15)	28

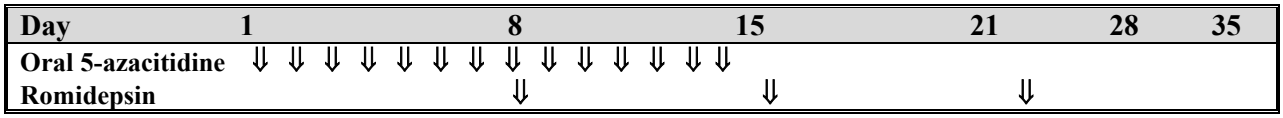
Level 2	200 (Days 1-14)	10 (Days 8 &15)	28
Level 3	300 (Days 1-14)	10 (Days 8 & 15)	28
Level 4	300 (Days 1-14)	14 (Days 8 & 15)	28
Level 5	300 (days 1-14)	14 (Days 8, 15 and 22)	35
Level 6	300 Days (1-21)	14 (Days 8, 15, 22)	35

Figure 2: Drug Administration Schema

Dose Levels -1 through 4



Dose Level 5



Dose Level 6

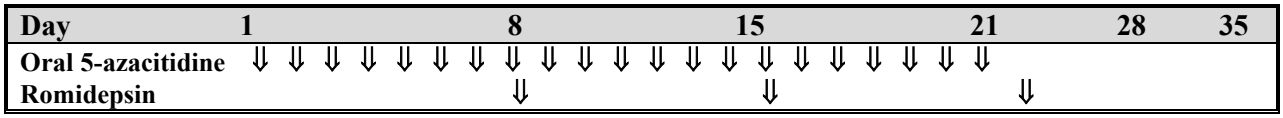


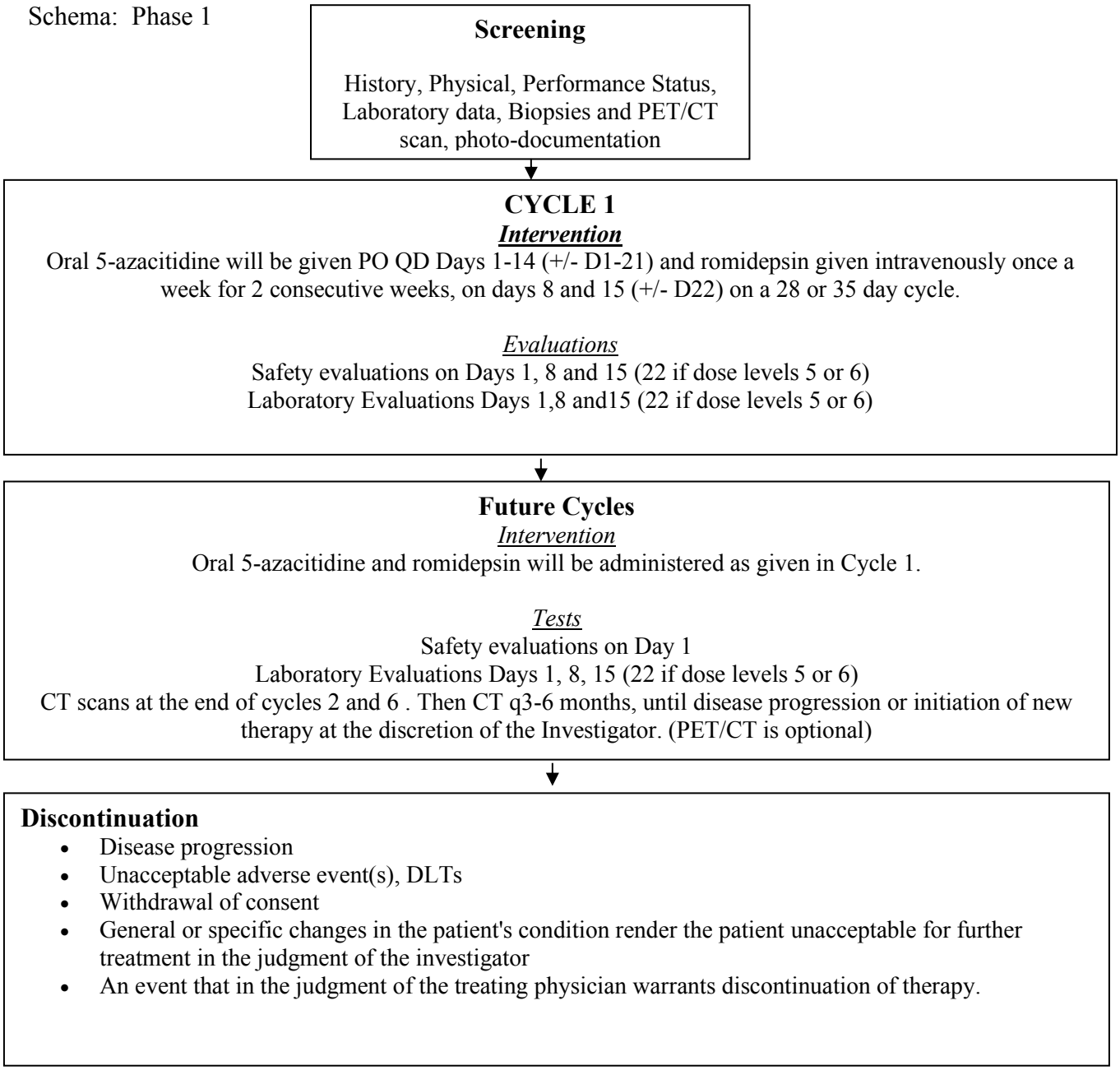
Table 3: Dose Escalation & De-Escalation Decision Rules:

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 additional patients experience DLT, proceed to the next dose level. • If 1 or more of this group experience DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose (MAD), and the dose level below is the MTD.

$\geq$2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered).
$\leq$1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

Figure 3: Study Plan Flow Chart

Schema: Phase 1



A. **Schema Phase II and Exploratory Cohort**

- Oral 5-azacitidine and romidepsin administered at the MTD of the combination to patients with T-Cell Lymphoma: oral 5-azacytidine at 300 mg (flat dose) Days 1-14 and romidepsin 14 mg/m2 on Days 8, 15 and 22 on a 35 day cycle.
- For Exploratory Cohort Only: Lymph node biopsy for determination of gene expression profile

↓

CYCLE 1

Intervention

Drugs are administered as outlined above

Evaluations

Safety evaluations on Days 1, 8, 15 and 22.
Laboratory Evaluations Days 1,8, 5 and 22
For Exploratory Cohort Only:
Optional lymph node biopsy on day 8 -21

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Future Cycles

Intervention

Both drugs given at the MTD

Tests

Safety evaluations on Day 1
Laboratory Evaluations Days 1, and each day of romidepsin
CT scans at the end of cycles 2 and 6. Then CT scans q3-6 months, until disease progression or initiation of new therapy.
(PET/CT is optional)

↓

Discontinuation

- Disease progression
- Unacceptable adverse event(s), DLTs
- Withdrawal of consent
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- An event that in the judgment of the treating physician warrant's discontinuation of therapy.

Table of Contents

1. OBJECTIVES

1.1. Phase I Primary Objectives

- Determine the maximum tolerated dose (MTD) and dose limiting toxicity (DLT) of the combination of oral 5-azacitidine and romidepsin.
- Evaluate the safety and toxicity of the combinations of oral 5-azacitidine and romidepsin.

1.2. Phase I Secondary Objectives

- Describe the maximum number of cycles received
- Describe the number of dose delays and dose reductions at the MTD
- Describe the anti-tumor activity of the combination
- Evaluate the overall response rate (ORR), progression free survival (PFS), and duration of response (DOR) of the study population.

1.3 Phase I Exploratory Objectives

- Evaluate pharmacodynamic markers of drug effect in paired tissue biopsies (pre- and post-treatment), including gene expression profiling and genome wide methylation status.
- Evaluate the pharmacokinetic profile for oral 5-azacitidine and romidepsin when given as a combination in cycle 1 at various time intervals as listed in figure 1.

1.4 Phase II Primary Objectives

- Estimate the ORR (complete + partial response) of the combination of oral 5-azacitidine and romidepsin in patients with relapsed/refractory T-Cell Lymphoma.

1.5 Phase II Secondary Objectives

- Estimate the DOR and PFS of the combination in patients with T-Cell Lymphoma.
- Estimate the overall survival of patients with T-Cell Lymphoma on study.
- Identify potential pre-treatment biomarkers of response based on GEP and/or methylation array) to clinical outcome.

1.6 Phase II Exploratory Objectives

- Evaluate pharmacodynamic markers of drug effect in paired tissue biopsies (pre- and post-treatment) including gene expression and genome wide methylation patterns.
- Evaluate pharmacokinetic profile for oral 5-azacytidine and romidepsin when given as a combination

1.7 Exploratory Cohort Primary Objectives

- Determine the overall response rates (complete responses + partial responses) of oral azacitidine and romidepsin in patients with GC-derived B-cell lymphomas.
- Determine Progression Free Survival (PFS) and Time to Treatment Failure (TTF)
- Determine the Duration of Response (DOR)
- Determine the safety and tolerability of oral azacytadine and romidepsin in patients with relapsed or refractory GC-derived B-cell lymphoid malignancies.

1.8 Exploratory Cohort Secondary Objectives

- Evaluate the pharmacokinetic (PK) profile of oral azacitidine and romidepsin.
- Evaluate and compare pharmacodynamic (PD) endpoints with PK endpoints. PD endpoints will include the evaluation of paired gene expression profiles of primary patient samples on study.

2. BACKGROUND

2.1 Background on Lymphoid Malignancies

The non- Hodgkin lymphomas (NHL) represent a heterogeneous group of malignancies. Under the rubric of lymphoma exist some of the fastest growing cancers known to science, (Burkett's lymphoma, lymphoblastic lymphoma/leukemia), as well as some of the most indolent (small lymphocytic lymphoma, follicular lymphoma, and marginal zone lymphoma). This remarkable diversity of biology imposes significant challenges. The first is on the pathologists who seek to understand the cell of origin and differentiate what are sometimes subtle differences between the related sub-types of disease. The second is on oncologists who seek to identify the best treatments for these subtypes, with the ever-increasing likelihood that our new understanding of the molecular pathogenesis of these diseases will result in an increase in new drugs for specific target populations.

In the United States, NHL represents 4-5% of all new cancer cases, and is the fifth leading cause of cancer death. In 2004, there were an estimated 54, 379 cases in the United States, and approximately 19,450 deaths.[2] Hodgkin lymphoma is a rare malignancy, with approximately 7,500 cases diagnosed yearly in the United States. An evaluation of the distribution of non-Hodgkin lymphoma subtypes was performed on the 114,548 cases of lymphoid neoplasms diagnosed between 1992-2001 in the Surveillance Epidemiology and End Results(SEER) registries, of which 87,666 were B-cell lymphoid neoplasms, 6,228 were considered T/Natural Killer (NK) cell neoplasms, and about 10,042 were attributed to Hodgkin lymphoma.[3] As a group, the diffuse, large-cell lymphomas (DLBCL) account for approximately one-third of all NHLs.

The peripheral T-cell lymphomas (PTCL) are a heterogeneous group of aggressive non-Hodgkin lymphomas (NHLs). They account for 10-15% of all newly diagnosed cases of NHL. The current annual prevalence of PTCL in the U.S. is estimated to be approximately 9,500 patients. PTCL- Not Otherwise Specified (NOS), a nodal subtype, is the most common T-Cell Lymphoma in the US and Europe. PTCL is often associated with a worse prognosis compared to its B-cell counterparts. Anthracycline based therapies typically provide a 5 year survival of less than 30%. Pralatrexate-azacitidine was the first drug approved for patients with relapsed/refractory PTCL[5]. The mature T-Cell Lymphomas also include the cutaneous T-Cell Lymphomas (CTCL), the 2 most common forms of which are mycosis fungoides (MF) and Sézary syndrome (SS). Between 2,000 and 3,000 new cases of CTCL occur in the United States each year. MF is categorized as limited stage (IA, IB, and IIA) or plaque/patche disease limited to skin, and advanced stage (IIB to IVB), characterized by cutaneous tumors and involvement of the blood, lymph

nodes, bone marrow, or visceral organs. SS is characterized by generalized erythroderma and abnormal lymphoid cells in the blood. Limited-stage disease may be effectively treated with skin-directed therapies including topical nitrogen mustard or psoralen plus ultraviolet A therapy. However, in patients with advanced disease, control is often short lived, and the disease is relentlessly progressive. Although response rates to cytotoxic chemotherapy range from 60 to 80% in patients with advanced disease, the median duration of response is usually measured in months. While agents with novel mechanisms of action have been pursued, including retinoids, interferon, monoclonal antibodies, and denileukin diftitox; none have been found to be curative. Romidepsin has single-agent clinical activity with significant and durable responses in patients with CTCL & PTCL[4].

GC derived lymphomas include GC-DLBCL and follicular lymphoma (FL), the two most common subtypes of lymphoma. Together they make up more than 50% of all lymphomas with an incidence of nearly 35,000 per year. Although the prognosis for these lymphoma subtypes is relatively good compared to other lymphoma subtypes, there are presently limited targeted treatment for these diseases. Salvage treatment options often include combination chemotherapy followed by autologous stem cell transplant, though many patients are not eligible for this therapy. The germinal center (GC) is a compartment of the lymph node that is responsible for generating high affinity antibodies via somatic hyper-mutation (SHM) and class switch recombination. Epigenetic modifiers such as EZH2, MLL, p300 and CBP, and the transcriptional repressor Bcl6 are essential to B-cell development, and are considered necessary for mutagenesis and silencing of tumor suppressor genes which facilitate SHM. This physiologic state is partly achieved by decreased acetylation and increased methylation of histones enforcing a transcriptionally repressed chromatin state. Mutations affecting these epigenetic and transcriptional modifiers have been identified as driving events in Germinal Center (GC) derived lymphomas.Given the high frequency of epigenetic derangements in germinal center (GC)-derived lymphomas, the use of histone deacetylase (HDAC) inhibitors and DNA methyltransferase (DNMT) inhibitors in combination are likely to have a synergistic effect, and this therapeutic response may be predicted by specific epigenetic disturbances that could be characterized by unique gene expression profiles.

2.2 Oral 5-azacitidine

2.2.1 Overview

Azacitidine is a pyrimidine nucleoside analog of cytidine that was first introduced for the treatment of AML in adults in 1976. (12) The antitumor effects of the drug are thought to be secondary to cytotoxicity as well as by hypomethylation of DNA. (13, 14, 15) Treatment of cells with azacitidine leads to inhibition of DNA, RNA, and protein synthesis. Once incorporated into the tRNA, azacitidine causes inhibition of tRNA methyltransferases, which interferes with tRNA methylation and defective acceptor function of transfer RNA. (16) In addition, azacitidine interferes with de novo thymidylate synthesis, adding to its cytotoxicity. Chan et al. were able to demonstrate that azacitidine causes a significant decrease in tumor methylation. (15) Biopsy specimens of 10 patients with EBV-associated tumors (nasopharyngeal carcinoma, Hodgkin lymphoma, and AIDS-associated diffuse small noncleaved lymphoma) were obtained before and after treatment with azacitidine at 75 mg/m2/day intravenously for 7 days. Using methylation-specific PCR, genomic sequencing, and immunohistochemistry, post-treatment specimens were found to have reversal of dense CpG methylation. (15)

Loss of DNA methylation is an important event in carcinogenesis. The role of CpG methylation in tumorigenesis includes the silencing of tumor suppressor genes, loss of expression of DNA repair enzymes, and loss of imprinting. There have been reports of decreased DNA methylation in colonic epithelium of patients with familial polyposis coli. Other examples illustrating the role of methylation

in cancer include the development of hepatocellular carcinoma in rats that are fed a methyl deficient diet, hypomethylation of H-ras and MYC genes in multiple tumors, and the finding of increased methylation of CpG islands of DNA in tumor models. (16)

2.2.2 Mechanism of Action

Azacitidine is a pyrimidine nucleoside analog of cytidine. Azacitidine is believed to exert its antineoplastic effects by causing hypomethylation of DNA and direct cytotoxicity on abnormal hematopoietic cells in the bone marrow. The concentration of azacitidine required for maximum inhibition of DNA methylation in vitro does not cause major suppression of DNA synthesis. Hypomethylation may restore normal function to genes that are critical for differentiation and proliferation. The cytotoxic effects of azacitidine cause the death of rapidly dividing cells, including cancer cells that are no longer responsive to normal growth control mechanisms. Non-proliferating cells are relatively insensitive to azacitidine.

2.2.3 Clinical Pharmacokinetics

The pharmacokinetics of azacitidine were studied in 6 MDS patients following a single 75 mg/m2 subcutaneous (SC) dose and a single 75 mg/m2 intravenous (IV) dose. Azacitidine is rapidly absorbed after SC administration; the peak plasma azacitidine concentration of 750 ± 403 ng/ml occurred in 0.5 hour. The bioavailability of SC azacitidine relative to IV azacitidine is approximately 89%, based on area under the curve. Mean volume of distribution following IV dosing is 76 ± 26 L. Mean apparent SC clearance is 167 ± 49 L/hour and mean half-life after SC administration is 41 ± 8 minutes.

Published studies indicate that urinary excretion is the primary route of elimination of azacitidine and its metabolites. Following IV administration of radioactive azacitidine to 5 cancer patients, the cumulative urinary excretion was 85% of the radioactive dose. Fecal excretion accounted for <1% of administered radioactivity over 3 days. Mean excretion of radioactivity in urine following SC administration of 14C-azacitidine was 50%. The mean elimination half-lives of total radioactivity (azacitidine and its metabolites) were similar after IV and SC administrations, about 4 hours.

2.2.4 Safety

The potential carcinogenicity of azacitidine was evaluated in mice and rats. Azacitidine induced tumors of the hematopoietic system in female mice at 2.2 mg/kg (6.6 mg/m2, approximately 8% the recommended human daily dose on a mg/m2 basis) administered IP 3 times per week for 52 weeks. An increased incidence of tumors in the lymphoreticular system, lung, mammary gland, and skin was seen in mice treated with azacitidine IP at 2.0 mg/kg (6.0 mg/m2, approximately 8% the recommended human daily dose on a mg/m2 basis) once a week for 50 weeks. A tumorigenicity study in rats dosed twice weekly at 15 or 60 mg/m2 (approximately 20% to 80% the recommended human daily dose on a mg/m2 basis) revealed an increased incidence of testicular tumors compared with controls.

Azacitidine is both mutagenic, and clastogenic in bacterial and/or mammalian cell systems and induces chromosomal aberrations in vitro.

Early embryotoxicity studies in mice revealed a 44% frequency of intrauterine embryonal death

(increased resorption) after a single IP injection of 6 mg/m2 (approximately 8% of the recommended human daily dose on a mg/m2 basis) azacitidine on gestation Day 10. Developmental abnormalities in the brain have been detected in mice given azacitidine on or before gestation Day 15 at doses of ~3-12 mg/m2 (approximately 4-16% the recommended
human daily dose on a mg/m2 basis). In rats, azacitidine was clearly embryotoxic when given IP on gestation Days 4 to 8 (postimplantation) at a dose of 6 mg/m2 (approximately 8% the recommended human daily dose on a mg/m2 basis), although treatment in the preimplantation period (on gestation Days 1-3) had no adverse effect on the embryos. Azacitidine caused multiple fetal abnormalities in rats after a single IP dose of 3 to 12 mg/m2 (approximately 8% the recommended human daily dose on a mg/m2 basis) given on gestation Day 9, 10, 11 or 12. The fetal anomalies included: CNS anomalies (exencephaly/encephalocele), limb anomalies
(micromelia, club foot, syndactyly, oligodactyly) and others (microphthalmia, micrognathia, gastroschisis, edema, and rib abnormalities). In this study azacitidine caused fetal death when administered 3 to 12 mg/m2 on gestation Days 9 and 10: average number of live animals per litter was reduced to 9% of control at the highest dose on gestation Day 9.

Administration of azacitidine to male mice at 9.9 mg/m2 (approximately 9% of the recommended human daily dose on a mg/m2 basis) daily for 3 days prior to mating with untreated female mice resulted in decreased in fertility and loss of offspring during subsequent embryonic and postnatal development. Treatment of male rats 3 times per week for 11 or 16 weeks at doses of 15-30 mg/m2 (approximately 20-40%, the recommended human daily dose on a mg/m2 basis) resulted in decreased weight of the testes and epididymides, and decreased sperm counts accompanied by decreased pregnancy rates and increased loss of embryos in mated females. In a related study, male rats treated for 16 weeks at 24 mg/m2 resulted in an increase in abnormal embryos in mated females when examined on Day 2 of gestation.

Through 18 May 2012, approximately 5,300 patients have been exposed to azacitidine during completed or ongoing Celgene-sponsored studies (n = 1,120 [992 with azacitidine injectable and 185 with azacitidine oral; 57 patients received both formulations]) or non-Celgene-sponsored studies (n = 4,190) worldwide.

The most commonly reported adverse reactions with azacitidine treatment were hematological reactions (eg, anemia, thrombocytopenia, neutropenia, leukopenia), GI events (eg, nausea, vomiting), and injection site reactions. Adverse reactions associated with IV azacitidine were similar in frequency and severity compared with SC azacitidine.

2.2.5 Efficacy in Non-Hodgkin Lymphoma

To date, there has been little experience with 5-azacitidine in patients with lymphoma. To date, 5-azacitidine has been approved by the US Food & Drug Administration for the treatment of:

- Myelodysplastic syndrome (MDS)
- Chronic myelomonocytic leukemia (CMMol)

This study represents the first to explore a hypomethylation inhibitor in patients with lymphoid malignancies. Dr. Cerchietti and colleagues conducted a phase I trial in patients with newly diagnosed diffuse large B-cell lymphoma. Eleven of the 12 patients enrolled were more than 60 years old when diagnosed, making them at at high risk for tumor recurrence after initial treatment. The patients were pretreated with azacitidine, in escalating doses, 8 days prior to initiation of 6 cycles of standard chemotherapy.

Of the 12 patients, 11 had a complete response and 10 remained in complete remission for up to 28 months. Side effects from pretreatment with azacitidine were minimal. Two patients who were treated with the maximum dose of azacitidine had dose-limiting toxicities.

The researchers conducted the clinical trial based on the results of their extensive preclinical experiments to determine the mechanisms by which lymphomas evade chemotherapy drugs. They found that compared with normal cells, all diffuse large B-cell lymphomas possess a high degree of aberrant DNA methylation, a process which "silences" certain genes, causing resistance to treatment.

Using diffuse large B-cell lymphoma cells and mice bearing human lymphoma xenografts, the researchers showed that DNA methyltransferase inhibitors are most effective if administered prior to chemotherapy, but not concurrently. They also found the gene *SMAD1* to be silenced in the unresponsive tumors.

When the researchers looked for *SMAD1* status in the biopsy specimens collected from patients enrolled in the phase I trial, they found that after treatment with azacitidine, there was a decrease in *SMAD1* methylation and increase in SMAD1 protein, providing proof of principle

2.3 Romidepsin:

2.3.1 Histone deacetylases and HDAC Inhibitors

Histone deacetylases (HDAC) are enzymes that catalyze the removal of acetyl groups from the lysine residues of various proteins, including histones and transcription factors. HDAC inhibitors can induce tumor cell growth arrest, differentiation, or apoptosis in vitro and inhibit tumor growth in animals[14-15]. The transcription of genes is regulated at least in part by acetylation of nucleosomal histones. The core nucleosomal histones are the most widely studied of the proteins that become acetylated following inhibition of HDAC activity[16]. In some cancer cells, there is an overexpression of HDACs, or an aberrant recruitment of HDACs to oncogenic transcription factors causing hypoacetylation of core nucleosomal histones. Hypoacetylation of histones is associated with a condensed chromatin structure and repression of gene transcription. Inhibition of HDAC activity allows for the accumulation of acetyl groups on the histone lysine residues resulting in an open chromatin structure and transcriptional activation.

2.3.2 Mechanism of Action of Romidepsin

Romidepsin (depsipeptide, FR901228, FK228, NSC 630176) is a relatively unique HDACi as it is a prodrug. Upon entering cells romidepsin is reduced to an active compound, capable of preferentially interacting with the zinc in the active site of the HDAC1, HDAC2 and HDAC3 (Class I)[17].

Romidepsin was granted full regulatory approval by the U.S. FDA for the treatment of relapsed CTCL. This approval is based on pooled data obtained from an NCI directed phase II trial of Romidepsin in CTCL and a registration directed trial by Celgene. This pooled analysis reported a response rate of 41% with a duration of response of approximately 14 months[18]. Ongoing studies continue to support the impressive activity of Romidepsin in PTCL with ORR of 31% and duration of responses in the range of 9 months.

Other HDAC inhibitors presently in clinical trials include b*elinostat* and panobinostat, both of which have demonstrated promising activity in T cell lymphomas. These and other data have clearly

established that HDACI as a class appear to have significant activity in T-cell lymphomas for reasons that are not clearly understood. HDAC inhibitors work through a myriad of different mechanisms including: (1) alteration in the expression of genes that regulate cell cycle including upregulation of p21/p27, and down regulation of Cyclin D; (2) ***acetylation*** of non-histone proteins including STAT-3, RelA/p65p53, HIF-1alpha, Hsp 90 in a way that may impair their function and influence cell growth and survival; (3) direct activation of apoptotic pathways by affecting the balance between the antiapoptotic proteins like BCL-2 and the proapoptotic proteins like BAX and BAK.[19] However, it has been difficult to assign any one or more of the above listed mechanisms as the mechanisms of action of HDAC inhibitors against any given tumor type, let alone in the T cell lymphomas. It is not even clear if histone acetylation is essential for the biological activity and apoptosis that is being seen in T cell lymphomas.

Interestingly, H3 acetylation can be demonstrated in tissue samples and mononuclear cells of patients who have been treated with HDACI, irrespective of their response to the therapy [19]. Nonetheless, attempts at understanding these mechanisms have involved gene expression profiling on paired tissue samples both pre- and post-treatment that has shown that only 5-10 % of the genome can be affected by HDACI. As many genes are regulated as are down-regulated following treatment with most HDACI. These changes have been shown to occur within a few hours of exposure (4 hours in one study)[20]. The genes that were consistently affected included genes that affected cell cycle (CCNDI, IGFI) apoptosis (septin10, TEF, SORBBS2), angiogenesis (GUCY1A1, ANGPT1) and immune modulation (LAIR1)[21]. QT-PCR was used to confirm that that the findings on gene array analysis were indeed biologically accurate, with a strong correlation between gene array and the PCR data. Tumor tissue specimens treated with various HDACI have shown an increase in histone acetylation, decreased vascularity and translocation of nuclear proteins like STAT-s which is associated with inactivation. [22]

2.3.3 Nonclinical Pharmacology of Romidepsin

Romidepsin is highly protein bound in plasma (92% to 94%) over the concentration range of 50 ng/mL to 1000 ng/mL with α1-acid-glycoprotein (AAG) being the principal binding protein.
Romidepsin undergoes extensive metabolism *in vitro* primarily by CYP3A4 with minor contribution from CYP3A5, CYP1A1, CYP2B6, and CYP2C19. At therapeutic concentrations, romidepsin did not competitively inhibit CYP1A2, CYP2C9, CYP2C19, CYP2D6, CYP2E1, or CYP3A4 *in vitro*.

2.3.4 Nonclinical Toxicology of Romidepsin

Romidepsin has been investigated in clinical trials. Major hematologic toxicity includes: anemia, leukopenia, lymphopenia and thrombocytopenia. Thus, blood counts are monitored during treatment and dose modifications may be necessary.

In addition, it was found that romidepsin may cause QT_c prolongation/ECG changes. While this has been exhaustively studied it is believed drug:drug interaction with select antiemetics may be responsible for the QTc prolongation. Nonetheless, obtaining baseline and periodic ECG (12-lead) and correcting electrolyte (potassium, magnesium, and calcium) abnormalities prior to and during treatment is highly recommended. T-wave and ST-segment changes have also been reported.

Carcinogenicity studies have not been performed with romidepsin. Romidepsin was not mutagenic *in vitro* in the bacterial reverse mutation assay (Ames test) or the mouse lymphoma assay. Romidepsin was not clastogenic in an *in vivo* rat bone marrow micronucleus assay when tested to the maximum tolerated

dose (MTD) of 1 mg/kg in males and 3 mg/kg in females (6 and 18 mg/m2 in males and females, respectively). These doses were up to 1.3-fold the recommended human dose, based on body surface area. Based on non-clinical findings, male and female fertility may be compromised by treatment with Romidepsin.

Please refer to the romidepsin Complete Investigators Brochure for detailed information.

2.3.5 Clinical Pharmacokinetics of Romidepsin

Romidepsin exhibited linear pharmacokinetics across doses ranging from 1.0 to 24.9 mg/m2 when administered intravenously over 4 hours in patients with advanced cancers. In patients with T cell lymphomas who received 14 mg/m2 of romidepsin intravenously over a 4-hour period on days 1, 8 and 15 of a 28-day cycle, geometric mean values of the maximum plasma concentration (Cmax) and the area under the plasma concentration versus time curve (AUC0-inf) were 377 ng/mL and 1549 ng*hr/mL, respectively.

Following 4-hour intravenous administration of romidepsin at 14 mg/m2 on days 1, 8 and 15 of a 28-day cycle in patients with T cell lymphomas, the terminal half-life (t1/2) was approximately 3 hours. No accumulation of plasma concentration of romidepsin was observed after repeated dosing.

2.3.6 Safety of Romidepsin

The safety of romidepsin was evaluated in 185 patients with CTCL in 2 single arm clinical studies in which patients received a starting dose of 14 mg/m2. The mean duration of treatment in these studies was 5.6 months (range: <1 to 83.4 months).

The most common drug-related adverse events in patients treated with romidepsin could be classified into 4 symptom complexes: gastrointestinal symptoms (diarrhea, nausea, anorexia, weight decrease, vomiting, and constipation), constitutional symptoms (fatigue, chills), hematologic abnormalities (thrombocytopenia, anemia) and taste disorders (dysgeusia, dry mouth). Most of the adverse experiences were manageable. In fact, most of the very common adverse events were reversible and could be managed using conventional supportive care for chemotherapy. On the whole, treatment with romidepsin was well tolerated.

Serious adverse reactions reported in > 2% of patients in Study 1 included infection, sepsis, and pyrexia. In Study 2, serious adverse reactions in > 2% of patients were infection, supraventricular arrhythmia, neutropenia, fatigue, edema, central line infection, ventricular arrhythmia, nausea, pyrexia, leukopenia, and thrombocytopenia. Most deaths were due to disease progression. In Study 1, there were two deaths due to cardiopulmonary failure and acute renal failure. In Study 2, there were six deaths due to infection (4), myocardial ischemia, and acute respiratory distress syndrome.

Discontinuation due to an adverse event occurred in 21% of patients in Study 1 and 11% in Study 2. Discontinuations occurring in at least 2% of patients in either study included infection, fatigue, QT prolongation, and dyspnea.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

2.3.7 Efficacy of Romidepsin

Positive results were reported in 71 patients with CTCL treated on the multicenter NCI study of romidepsin administered as a 4-hour infusion on days 1, 8, and 15 of a 28-day cycle with a starting dose of 14 mg/m^2[19]. The ORR was 34%, with a CR observed in four patients, a PR in 20, and SD in 26. The median time to progression for patients with a major response (CR or PR) was 15.1 months.

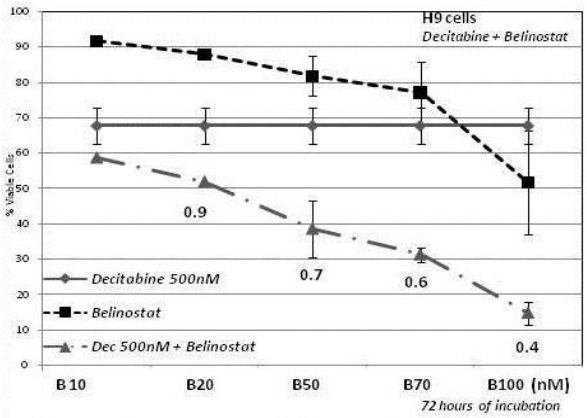
Very encouraging responses have also been reported in patients with peripheral T-cell lymphoma (PTCL). ORR of 31%, as a single agent in 48 patients, including four CR and 11 PR [25]. The overall median duration of response for all patients was 9 months.

In 2011, the US FDA approved romidepsin for patients with relapsed/refractory PTCL.

2.4 Rationale for the Study

2.4.1 Hypothesis and Preliminary Data

Targeting HDAC's in patients with TCL has proven to be a valuable therapeutic approach [7]. Vorinostat became the first HDACi approved for the treatment of cancer, for patients with relapsed and refractory CTCL in 2006, followed in 2009 by the approval of romidepsin and expanded in 2011 to include patients with relapsed and refractory PTCL. The promising experience with HDACi in T-cell lymphoma, driven by empirical observations, established that targeting the 'epigenome' was a viable treatment strategy for select sub-types of lymphoma, albeit, only effective in about one-third of all TCL patients [8, 9]. Experiences from myeloid leukemia and myelodysplastic syndrome have suggested that combinations of epigenetically targeted drugs, including HDACi plus hypomethylating agents (HoMe), may have more therapeutic value than either of these agents alone. In addition***, HDACi were shown to decrease DNMT-1***, leading to reduced genomic methylation and gene activation [15]. These data suggest that the overlapping influences HDACi and HoME on the epigenome could be exploited in rational combinations.



H9	(uM)	
	RRR	
(nM)	D 0.5	D 1
B 50	0.6	0.6
B 70	0.6	0.5
B 100	0.4	0.5
R 0.5	0.9	0.9
R 1	0.7	0.7
R 2	0.3	0.2
L 4	0.6	0.5
L 5	0.6	0.4
L 7	0.3	0.3
S 600	0.6	0.6
S 800	0.4	0.4
S 1000	0.3	0.5

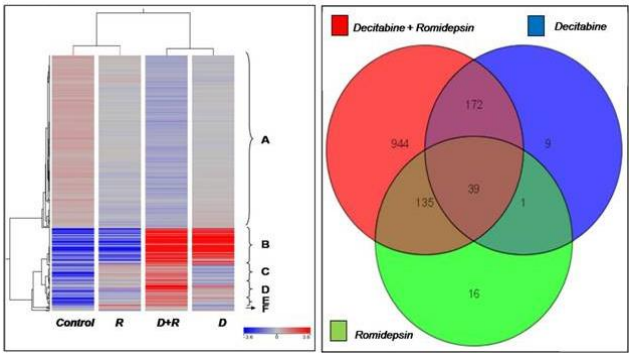
HH	(uM)	
	RRR	
(nM)	D 1	D 10
B 20	_	0.9
B 50	_	0.7
B 100	_	0.8
R 0.5	_	0.9
R 1	_	0.6
R 2	_	0.1
L 6	0.8	0.6
L 8	0.7	0.7
L 10	0.5	0.7
S 600	0.8	0.8
S 800	0.8	0.7
S 1000	0.7	0.7

To date we have evaluated these two drug classes in combination in a variety of preclinical models of T-cell lymphoma, [16], and have explored the influence of the individual drugs and their combination on gene expression in different sub-types of T-cell lymphoma. These data revealed substantial synergism between the hypomethylating agents (*decitabine and 5-azacitidine)* and various *HDACi including romidepsin, belinostat, vorinostat and panobinostat* with synergy coefficients ranging between 0.1 and 0.9 (**see Figure below**) [17]. While the synergy seen here was observed following a modest (~12 hr) lead in of the HoMe agent, it suggests a class effect between the different HDACi and the HoMe agent, where the $IC_{10\text{-}20}$ of each drug against the specific cell line was used in the combination. These data also underscore the fact that there are many variables that determine

this degree of synergy, including the particular drug-drug combination, drug concentration, schedule and duration of exposure.

We recently showed that the combination of romidepsin and the HoMe agent decitabine reversed the malignant phenotype of all T-cell lymphoma cell lines tested, with each individual drug having only a partial effect on the expression signature (**Figure 3**) [17]. The GEP data also demonstrated that while common sub-sets of genes could be affected, the greatest number of 'unique' genes perturbed were seen in those cells treated with the combination, where nearly 1000 unique genes were altered, strongly supporting the hypothesis that the synergism seen at the cellular level and in the *in vivo* model, was also seen at the molecular level. The Venn diagram below illustrates the principle: the effects of the two drugs alone are largely different (only 39 genes modified in common by all the treatment groups), while most of the effects induced by the single agent treatment are maintained in the combination group (174 genes out of 191 for romidepsin and 211 genes out of 221 for decitabine). Interestingly, an additional 944 unique genes (i.e. genes not affected by the single agents) are modulated only by the combination treatment, strongly supporting the hypothesis of 'molecular synergism'.

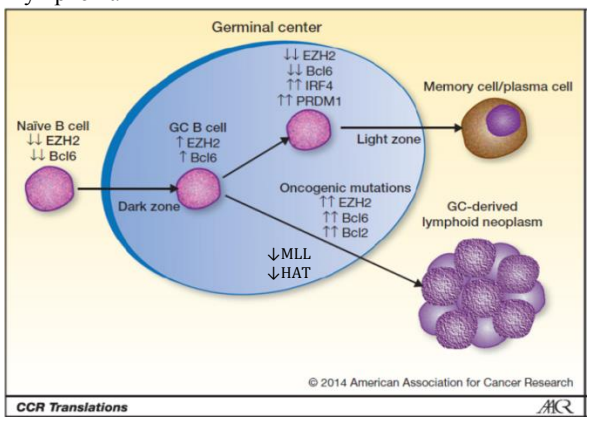
Figure 1. Cytotoxicity curves in H9 and HH following exposure to decitabine and HDAC inhibitor . The tables list synergy coefficients for the specified combination. [R=romidepsin;B=belinostat;S=SAHA; L=LBH589/panobinostat.] In subsequent experiments with a SCID Beige Xenograft Mouse Model of HH-CTCL, mice were treated i.p. for 3 cycles with decitabine, belinostat and the combination. Statistically significant tumor growth inhibition was observed in the combination cohort compared to all the others.



Collectively, these data suggest that dual targeting of the epigenome in T-cell lymphoma may represent an incremental improvement in our ability to treat the disease compared to single agent (or class) approaches predicated on HDAC inhibitors alone.

1. Of patients with Germinal Center (GC)-DLBCL treated with standard therapy, 30% will relapse (12) and less than half of these patients will be eligible for intensive salvage therapy (13). There is a need to improve therapeutic options as there are limited-to-no targeted approaches for salvaging patients with refractory GC-DLBCL. As we have reviewed in *Clinical Cancer Research* (14)(**Fig 4**), new discoveries into the molecular pathogenesis of this disease have created enthusiasm around the development of precision targeted strategies (**Fig 5**). Although it is known that mutations in BCL6, EZH2, histone acetyltransferases (HATs) and other epigenetic modifiers all contribute to silencing of tumor suppressors, it is not known what effects simultaneous epigenetic dysregulation have on the biology of GC-lymphomas, or their responsiveness to targeted treatment. *Given the clustering of epigenetic mutations in GC-derived lymphomas, the possibility of directly targeting HATs combined with targeting of complimentary epigenetic machinery may serve as a promising future treatment paradigm for this specific*

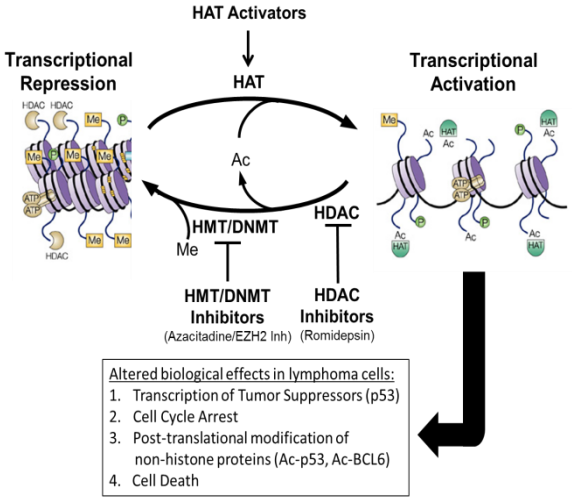
Figure 4: Schematic of derangements in Germinal Center Lymphoma



*disease entity (**Fig 5**)*(14). Past studies have focused on individually targeting these factors, but not studying them together as a whole. Our laboratory has made several pivotal observations regarding the targeting of epigenetic operations in DLBCL. Using a translational approach, we have taken direct observations from the laboratory and applied them to clinical trials. Some examples include:We have established that targeting the Bcl6 : p53 axis with HDAC and Sirtuin inhibitors inactivates Bcl6 and activates p53 leading to clinically meaningful responses in patients with lymphoma (Amengual Blood 2013)(8) (**Fig 6, 7**).

2. We have demonstrated that combinations of DNMT and HDAC inhibitors are synergistic in DLBCL and led to modulation of *SMAD1* and *DNMT3A* which are known to influence chemotherapy resistance (Kalac Blood 2011)(10) (**Fig 8**).

Figure 5: Schematic of epigenetic modulation in GC-lymphomas



Modulating Acetylation of the Bcl6 : p53 Axis. Small molecule inhibitors of HDACs help maintain transcriptionally active chromatin, theoretically allowing for expression of tumor suppressor genes (22). Three HDAC inhibitors, vorinostat (23, 24), belinostat (25), and romidepsin (26, 27), have been all approved by the FDA for the treatment of T-cell lymphoma. Despite approval in T-cell lymphoma, single agent HDAC inhibitors have demonstrated limited activity in relapsed DLBCL (28). *I have demonstrated that the combination of niacinamide (N), a sirtuin inhibitor, and HDAC inhibitors (romidepsin (R), vorinostat (V), belinostat (B), or panobinostat (P)) leads to synergistic cytotoxicity in GC-DLBCL but not ABC-DLBCL (**Fig 6**)*[Amengual, et. al (8)]. Figure 3 represents the synergy co-effiecients of combinations of 4 different HDAC inhibitors with niacinamide in 8 DLBCL cell lines. Red boxes indicate synergistic interactions. As represented here, the GC-DLCBL cells are more sensitive to the combination. Our group developed a genetically engineered mouse (lambda-*myc* crossed with mCherry fluorescent CD19) which develops spontaneous B-cell lymphoma. This mouse model expresses similar levels of Bcl6 and p53 as the GC-DLCBL cell lines (29). Following exposure to romidepsin and niacinamide, mouse lymphoid tissue demonstrated acetylation of both Bcl6 and p53 measured by immunoprecipitation as well as modulation of downstream targets BLIMP1 and p21 measured by immunoblot (**Fig 7**). The combination was well tolerated in mice and led to increased tumor growth delay compared to either drug alone as measured by *in vivo* imaging (**Fig 7**). These findings demonstrated for the first time the potential clinical application of previous published data cited above. Translating these results, we conducted a phase I clinical study of vorinostat and niacinamide in relapsed/refractory lymphoma which demonstrated an ORR of 24% (NCT00691210). This proof-of-principle study demonstrates a potential role of combined HDAC inhibition therapy in B-cell lymphomas (8). In addition, it suggests that the cumulative epigenetic derangements of GC-DLBCL may be amenable to targeted therapy through modulation of the acetylation state.

3.

Combined targeting with HDAC and DNMT inhibitors. Converse to the effects of acetylation, DNA hypermethylation can lead to transcriptional silencing of tumor suppressor genes and resistance to chemotherapy. Unlike HATs, mutations in DNMTs have not been identified in DLBCL, however they have been found to be overexpressed (DNMT1 by 48%) and this has been linked to clinical stage(30). Pre-clinical studies have shown that histone deacetylation and DNA methylation are linked, and cooperate to inactivate a host of tumor suppressor genes (31). *We have demonstrated synergy between HDAC and DNMT inhibitors in T- and B-cell lymphomas(9, 10). The combination of decitabine with panobinostat demonstrated potent*

synergy in vitro, which was validated in a xenograft model of GC-DLBCL where the combination led to marked

Figure 6: Combination of niacinamide with HDAC inhibitors is synergistic in GC-DLBCL

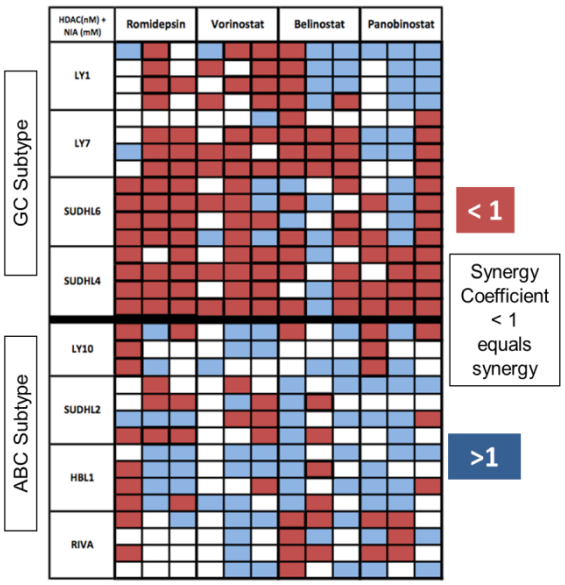


Figure 7: Combination of niacinamide with romidepsin leads to acetylation of Bcl6 and p53 in mice and decreased tumor burden

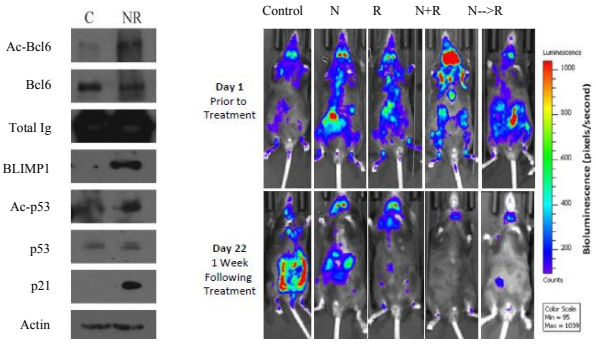
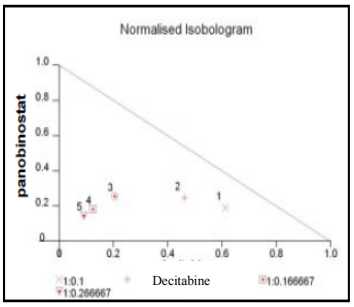
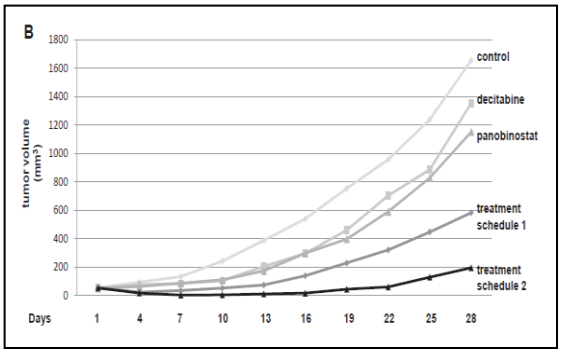


Figure 8: Panobinostat is synergistic with decitabine in preclinical models of GC- DLBCL

	RRR	CI
1.25nM / 0.12μM	0.8	0.8
2.5nM / 0.25μM	0.9	0.7
5nM / 0.5μM	0.6	0.4
6nM / 1μM	0.5	0.3
7.5nM / 2μM	0.4	0.2





*tumor growth delay compared to either agent alone (**Fig 8**)*. This combination also led to unique effects on gene expression, where treatment with panobinostat led to an increase in *SMAD1* and *DNMT3A* which may explain the synergistic benefit of DNMT and HDAC inhibition(10). This concept has been recently translated to the clinic in a study of azacitadine in combination with romidepsin for the treatment of patients with relapsed/refractory lymphomas (NCT 01998035)(32) and as a method to re-sensitize DLBCL to chemotherapy (NCT 0243536) (33). These findings underscore that modulating both acetylation and methylation has a path to the clinic for GC-DLCBL.

2.4.2 Phase I Experience and Determination of MTD

Twenty-six patients were enrolled in the Phase I study and were evaluable for toxicity. Median age was 51 yrs (23-79) and 58% were male. The median number of prior therapies was 6.5 (range 1-18). Histologies included HL (N=12), DLBCL (N=5), follicular lymphoma (N=3), ATLL (N=2), CTCL (N=2), cutaneous ALCL (N=1), T-ALL (N=1). The entry dose in cohort 1 was azacytidine at 100 mg day 1 to 14 with romidepsin 10 mg/m2 day 8, 15 every 21 days with no DLT detected. There were 2 DLTs in cohort 2 (azacytidine 200mg day 1 to 14 with romidepsin 10 mg/m2 day 8, 15 every 21 days) consisting of 1 Grade 3 thrombocytopenia and 1 Grade 3 pleural effusion. In cohort 3, 3 additional patients were evaluated using the every 28 days schedule at the same dose level and no thrombocytopenia and/or other DLTs were observed. Dose escalation was continued until 2 DLT were observed in cohort 7 (azacytidine 300mg day 1 to 21 with romidepsin 14mg/m2 day 8, 15, 22 every every 35 days which resulted in 1 Grade 4 thrombocytopenia and 1 Grade 4 neutropenia. This dose level was declared maximum administered dose (MAD). Patients dosed at the maximum tolerated dose (MTD) of azacytidine 300 mg day 1 to 14 and romidepsin 14 mg/m2 day 8, 15, 22 every 35 days did not experience any toxicities.

The toxicities reported in >10% of patients included: grade 3/4 toxicities represented by febrile neutropenia (19.2%) and thrombocytopenia (19.2%); grade 2 toxicities represented by back pain (7.7%), fatigue (11.5%), nausea (34.6%), vomiting (19.2%), pain (7.7%) and peripheral sensory neuropathy (7.7%) and grade 1 toxicities including fatigue (38.5%), nausea (23%), diarrhea (19.2%), malaise (19.2%), vomiting (19.2%), anorexia (15.4%), pain (15.4%), constipation (11.5%), cough (11.5%), fever (11.5%), general disorders and administration site complains (11.5%), dyspnea (7.7%), hypokalemia (7.7%), insomnia (7.7%), oral mucositis (7.7%) and pruritus (7.7%).

Twenty-six patients were enrolled, of which 23 were evaluable for response. The overall response rate was 30%. Interestingly, as hypothesized, the ORR among the B- and T-cell patients were dramatically different, albeit the number of T-cell patients was quite small. For instance, there were 18 patients with B-cell lymphoma, of which only 3 responded, giving an ORR of 17% among the B-cell lymphoma, which did not include any complete remission. Among the 5 T-cell patients, 4 responded, for an ORR of 80%, and 2 of the 4 responses were complete remissions. These responses included: (1) a patients with refractory T-ALL who achieve a firs CR on study allowing a MUD-allogeneic stem cell transplant; (2) a patients with drug resistant CD8+ cutaneous cytotoxic T-cell lymponoma who attained a CR despite relapsing off pralatrexate; (3) a patient with HTLV-1 ATLL who attained a CR, and (4) a patient with CTCL who attained a major PR. One patients with drug resistant tumor MF failed to respond. We believe these observations, although early, are consistent with the laboratory data and our hypothesis, that this regimen is likely to have a highly lineage specific activity in patients with PTCL and CTCL.

Moreover, we believe the totality of the data support continued study of this promising combination.

To date there have been 7 patients enrolled with either follicular or DLBCL. Five of these patients were evaluable for response and 1 patient had a partial response (20%) and a second had stable disease. There is an emerging strong rational to evaluate patients with germinal center derived B-cell lymphoma with combination epigenetic therapy as will be discussed below.

3. PATIENT SELECTION

3.1 Inclusion Criteria for Lymphoma

3.1.1 Phase I: Patients must have histologically confirmed relapsed or refractory non-Hodgkin lymphoma or Hodgkin lymphoma (WHO criteria), with no accepted curative options.
Phase II: Patients with untreated, relapsed or refractory T-cell lymphoma, including patients with central nervous system (CNS) involvement or lymphomatous meningitis are allowed on study.
Exploratory Cohort: Patients with histologically confirmed relapsed or refractory Germinal Center (GC)-derived B-Cell Lymphoma (diffuse large B-cell (DLBCL) and follicular lymphoma (FL)) defined by the WHO and Hans criteria with no accepted curative options. (Appendix 5)

3.1.2. There is no upper limit for the number of prior therapies. Patients may have relapsed after prior autologous or allogeneic stem cell transplant.

3.1.3 Evaluable Disease in the Phase I, and measurable disease as defined in Section 11.1 for the Phase II.

3.1.4 Age ≥18 years.

3.1.5 ECOG performance status ≤2

3.1.6 Patients must have adequate organ and marrow function as defined below:

- absolute neutrophil count ≥1,000/dL
- platelets ≥75,000 total (≥50,000 if bone marrow involvement)
- bilirubin ≤1.5X institutional limits
- AST(SGOT)/ALT(SGPT) ≤2.0X institutional upper limit of normal
- Serum creatinine within normal institutional limits
  OR
- Creatinine clearance ≥50 mL/min/for patients with creatinine levels above institutional normal.

3.1.7 Negative urine or serum pregnancy test for females of childbearing potential

3.1.8 All females of childbearing potential must use an effective barrier method of contraception (either an IUDC or double barrier method using condoms or a diaphragm plus spermicide) during the

treatment period and for at least 1 month thereafter. Male subjects should use a barrier method of contraception during the treatment period and for at least 3 months thereafter. Female subjects should avoid the use of estrogen-containing contraceptives, since Romidepsin may reduce the effectiveness of estrogen-containing contraceptives.

3.1.9 Ability to understand and the willingness to sign a written informed consent document.

3.2 Exclusion Criteria

3.2.1 Prior Therapy

- ❖ Exposure to chemotherapy or radiotherapy within 2 weeks prior to entering the study or those who have not recovered from adverse events due to agents administered more than 2 weeks earlier.
- ❖ Systemic steroids that have not been stabilized (≥ 5 days) to the equivalent of ≤10 mg/day prednisone prior to the start of the study drugs.
- ❖ No other concurrent investigational agents are allowed.

3.2.3 History of allergic reactions to Oral 5-azacitidine or Romidepsin.

3.2.4 Uncontrolled intercurrent illness including, but not limited to, ongoing or active infection, symptomatic congestive heart failure, unstable angina pectoris, cardiac arrhythmia, or psychiatric illness/social situations that would limit compliance with study requirements.

3.2.5 Pregnant women

3.2.6 Nursing women

3.2.7 Active concurrent malignancy (except non-melanoma skin cancer or carcinoma in situ of the cervix). Patients whose lymphoma has transformed from a less aggressive histology remain eligible.

3.2.8 Patients known to be Human Immunodeficiency Virus (HIV)-positive

3.2.9 Patients with active hepatitis A, hepatitis B, or hepatitis C infection.

3.2.10 Concomitant use of CYP3A4 inhibitors (see Appendix 2)

3.2.11 Any known cardiac abnormalities such as:

- Congenital long QT syndrome
- QTc interval ≥ 500 milliseconds;
- Patients taking drugs leading to significant QT prolongation (See Appendix 1: Medications That May Cause QTc Prolongation)
- Myocardial infarction within 6 months of C1D1. [Subjects with a history of myocardial infarction between 6 and 12 months prior to C1D1 who are asymptomatic and have had a negative cardiac

risk assessment (treadmill stress test, nuclear medicine stress test, or stress echocardiogram) since the event, may participate];

- Other significant ECG abnormalities including 2nd degree atrio-ventricular (AV) block type II, 3rd degree AV block, or bradycardia (ventricular rate less than 50 beats/min);
- Symptomatic coronary artery disease (CAD), *e.g.,* angina Canadian Class II-IV (see Appendix 3) In any patient in whom there is doubt, the patient should have a stress imaging study and, if abnormal, angiography to define whether or not CAD is present;
- An ECG recorded at screening showing evidence of cardiac ischemia (ST depression of ≥2 mm, measured from isoelectric line to the ST segment). If in any doubt, the patient should have a stress imaging study and, if abnormal, angiography to define whether or not CAD is present;
- Congestive heart failure (CHF) that meets New York Heart Association (NYHA) Class II to IV definitions (see Appendix 4) and/or ejection fraction <40% by MUGA scan or <50% by echocardiogram and/or MRI;
- A known history of sustained ventricular tachycardia (VT), ventricular fibrillation (VF), Torsade de Pointes, or cardiac arrest unless currently addressed with an automatic implantable cardioverter defibrillator (AICD);
- Hypertrophic cardiomegaly or restrictive cardiomyopathy from prior treatment or other causes;
- Uncontrolled hypertension, *i.e.,* blood pressure (BP) of ≥160/95; patients who have a history of hypertension controlled by medication must be on a stable dose (for at least one month) and meet all other inclusion criteria; or
- Any cardiac arrhythmia requiring an anti-arrhythmic medication (excluding stable doses of beta-blockers)

3.3 Inclusion of Women and Minorities

Both men and women of all races and ethnic groups are eligible for this trial.

4. REGISTRATION PROCEDURES

4.1 General Guidelines

Eligible patients will be enrolled on study by the study team.

Following enrollment, patients should begin protocol treatment within 14 days. Issues that would cause treatment delays should be discussed with the Principal Investigator. If a patient does not receive protocol therapy within 14 days of enrollment, the patient's enrollment on the study may be canceled, at the discretion of the PI.

4.2 Screening

All potential study patients will be screened and eligibility determined prior to enrollment. Unless otherwise specified, the following procedures and evaluations will be performed as noted in the study calendar (Section 10) prior to the start of study drugs (cycle 1, dose 1):

1) Obtain written informed consent and privacy authorization prior to initiating any protocol-required procedure that is not considered standard of care (See Section 4.3)

2) Review eligibility criteria

3) Review medical chart for past medical/surgical history

4) Record medications and prior treatment regiments

5) Record response to prior treatment regimen(s)
6) Document histopathology from:
   (i) Original diagnosis
   (ii) Tumor biopsy in the relapsed setting (if applicable)

7) Documentation of known measurable disease parameters by the following procedures:
   - CT of neck, chest, abdomen, and pelvis (PET/CT optional)
   - Skin exam
   - Other imaging techniques documenting disease site other than neck, chest, abdomen, and pelvis, if applicable

8) Obtain a 12-lead electrocardiogram (ECG) and calculate the QTc interval.

9) Perform a comprehensive physical examination

10) Assess and record ECOG Performance Status

11) Local laboratory: Collect blood for hematology (CBC with differential counts), chemistry (Na, K, Cl, HCO3, BUN, creatinine, glucose, calcium, magnesium, including serum β-human chorionic gonadotropin [β-hCG] pregnancy test for women who are not postmenopausal or surgically sterile [within 7 days prior to cycle 1, dose 1 and again within 24 hours prior to first dose of the study drugs.]), liver function tests (total protein, Albumin, AST, ALT, total bilirubin, direct bilirubin, alkaline phosphate).

12) Calculate creatinine clearance using the glomerular filtration rate (GFR) according to the Cockroft and Gault Equation, only if screening serum creatinine is > 1.5 mg/dL: GFR* = (140 – age [years]) x actual body weight (kg)72 x serum creatinine
   *For female patients, multiply by 0.85

13) For Exploratory Cohort Only: A lymph node, core-needle biopsy, or bone marrow biopsy will be performed during the screening period for evaluation of gene expression profiling .

4.3 Informed Consent

4.3.1 Study Informed Consent

Study personnel must obtain documented consent from each potential patient prior to entering in a clinical study. Consent must be documented on the IRB approved consent form by obtaining the dated signature both of the patient and of the investigator conducting the consent discussion. If the patient is unable sign the consent form, then oral consent, attested to by the dated signature of an impartial witness (someone not involved with the conduct of the study), is the required alternative.

If the patient is illiterate, an impartial witness should be present during the entire informed consent reading and discussion. Afterward, the patient should sign and date the informed consent, if capable. The impartial witness should also sign and date the informed consent along with the individual who read and discussed the informed consent (i.e., study staff personnel).

The information from the consent form should be translated and communicated to the subject in language understandable to the subject. Consent forms will be available in English and Spanish. When the study participant is non-English and non-Spanish speaking, the consent form must be read accurately in its entirety by a qualified professional translator. The translator will provide a written statement indicating that the consent form has been accurately translated from the accompanying English version, and that the study participant consents to participation. The professional translator will sign the consent form as an impartial witness.

A copy of the signed and dated consent form should be given to the patient before participation in the study.

Patients may undergo study screening tests prior to giving written informed consent provided that these tests are considered part of standard care.

The initial informed consent form and any subsequent revised written informed consent form, and written information will receive the IRB approval. The patient or his/her legally acceptable representative will be informed in a timely manner if new information becomes available that may be relevant to the patient's willingness to continue participation in the trial. The communication of this information will be documented.

4.3.2Consent and Use of Tissue Specimens for Research

Patients who choose to participate in the optional biopsies will undergo a tissue biopsy pretreatment and a second biopsy between Day 8 and 21 of cycle 1. The investigator or designee is responsible for explaining and verifying the subject's consent before obtaining any biopsy. It will be explained to the patient that allowing the lymph node sample is encouraged, but optional, and participation in the associated clinical study is not dependent upon giving this sample.

4.4 Registration Process

To register a patient, the following documents should be completed by a member of the research staff and delivered to the study coordinator:

- Eligibility Screening Worksheet
- Copy of required laboratory and imaging tests
- Signed patient consent forms
- HIPAA authorization form

The Study Coordinator will verify eligibility. To complete the registration process, the Coordinator will:

- Assign a patient study number
- Register the patient on the study
- Confirm registration with the principal investigator

5. TREATMENT PLAN

5.1 Agent Administration

5.1.1Dose Escalation Scheme

Cohorts of 3 patients will be administered oral 5-azacitidine and romidepsin starting at a dose of 100 mg for oral 5-azacitidine and 10 mg/m^2 for romidepsin. Dose escalations will then commence for each agent individually with a maximum oral 5-azacitidine dose of 300 mg and a romidepsin dose of 14 mg/m^2 representing the highest dose cohort. The dose levels to be considered are outlined in the escalation schema in Table 2. The rules for dose escalation or de-escalation are outline in table 3.

Table 1: Regimen Description

REGIMEN DESCRIPTION					
Agent	***Premedications; Precautions***	***Dose***	***Route***	***Schedule***	***Cycle Length***
Oral 5-azacitidine	Anitemetic	300 mg (flat dose)	PO	Days 1-14	*35 days*
Romidepsin	Antiemetic	14 mg/m2	IV over 4 hours (Administered first during cycle 1)	Days 8, 15, 22 (+/- D 22)	
** *Doses as appropriate for assigned dose level.*					

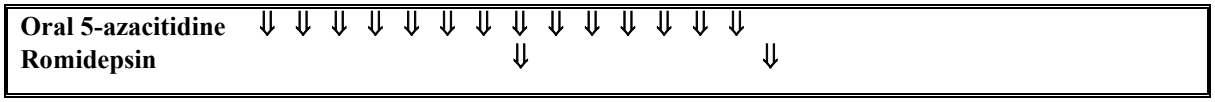
Table 2: Dose Escalation Scheme for the Combination of Oral 5-azacitidine and Romidepsin

Dose Escalation Schedule			
Dose Level	Dose		
	Oral 5-azacitidine (Flat dose [mg])	*Romidepsin (mg/m2 rounded to 14mg/m2)*	*Cycle Length*
Level -1	100 (Days 1-14)	10 (Day8)	28
Level 1	100 (Days 1-14)	10 (D8 & 15)	28
Level 2	200 (Days 1-14)	10 (Day 8 &15)	28
Level 3	300 (Days 1-14)	10 (Day 8 & 15)	28
Level 4	300 (Days 1-14)	14 (day 8 & 15)	28
Level 5	300 (days 1-14)	14 (Days 8, 15 and 22)	35
Level 6	300 Days (1-21)	14 (Days 8, 15, 22)	35

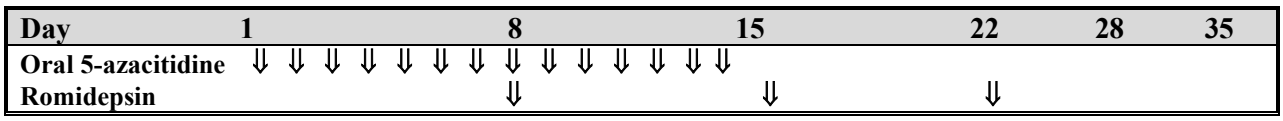
Figure 2: Drug Administration Schema

Dose Levels -1 through 4

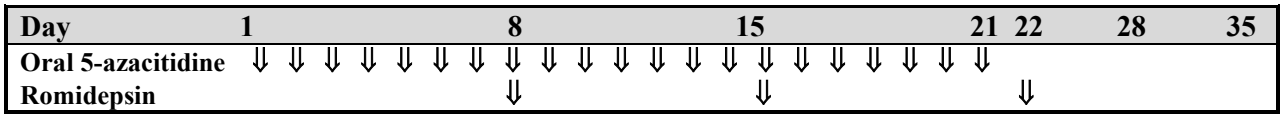
Day	**1**	**8**	**15**	**22**	**28**



Dose Level 5



Dose Level 6



The objective for the dose escalation scheme for Part I is to evaluate the safety and tolerability of various doses of combined oral 5-azacitidine and HDAC inhibition by romidepsin.

Phase II will evaluate the efficacy (ORR) of the MTD of oral 5-azacitidine and romidepsin from Part I in patients with T-Cell Lymphoma. (See figure 2 for study plan flow chart)

5.1.2Regimen Description (Phase 2)

Treatment will be administered on an outpatient basis. Patients will be treated with oral 5-azacytidine and romidepsin at the MTD (i.e the Recommended Phase 2 Dose, or RP2D). The treatment will be administered as follows: oral 5-azacytidine 300 mg (flat dose) Days 1-14 and romidepsin 14 mg/m2 on Days 8, 15, and 22 on an every 35 day cycle. All patients receiving romidepsin will receive potassium and magnesium supplementation as recommended in the package insert of the product. In all cycles romidepsin will be administered first over 4 hours. Reported adverse events and potential risks for oral 5-azacitidine and romidepsin are described in Section 7. Appropriate dose modifications are described in Section 6. No investigational or commercial agents or therapies other than those described may be administered with the intent to treat the patient's malignancy.

5.1.3 Required Blood Parameters and Other Investigations Prior to Each Treatment (Except Cycle 1 Day 1)

Before the start of each treatment, patients should be reassessed and the following criteria must be fulfilled:

- ANC > 1000/dL
- Platelet count ≥ 50, 000/dL
- Serum creatinine concentration ≤ 2.0 × ULN or ≤ baseline, or creatinine clearance >50 ml/min
- AST (SGOT) and ALT (SGPT) ≤2.0 × ULN or ≤3.0 × ULN in presence of demonstrable liver metastases
- Bilirubin concentration ≤2.0 × ULN
- Serum potassium is ≥ institutional LLN and magnesium is ≥ laboratory LLN (supplements must be given to patients whose potassium is < laboratory LLN and/or whose magnesium is < laboratory LLN and serum electrolytes must be rechecked prior to romidepsin administration).
- Recovery of any drug-related non-hematological toxicity to Grade 1 or less, unless otherwise indicated

- ECG schedule as specified in Section 5.1.8.2.

5.1.4 Guidelines for Study Drug Administration

- Oral 5-azacitidine will be taken between the hours of 8 and 10 am daily (patient will be required to maintain a drug log recording side effects and time of dosage) on Days 1-14
- Romidepsin will be administered over 4 hours through either a peripheral or central venous catheter on Days 8, 15, 22 of a 35 day cycle. It will be administered within 6 hours of the oral dose. On the day of PK sampling in Cycle 1, the azacitidine will be taken with romidepsin. A dose of the anti-emetic will be administered in accordance with the package insert of romidepsin.
- Administration of the study drugs will be allowed within 48 hours of the scheduled dates.
- Missed doses will not be made up.

5.1.5 Maximum Tolerated Dose

The dose-escalation rules and determination of the maximum tolerated dose (MTD) are outlined in Tables 2 and 3. Patients will be enrolled to receive oral 5-azacitidine and romidepsin in sequentially escalating doses until a maximum tolerated dose of the combination has been defined. The maximum tolerated dose of oral 5-azacitidine in combination with romidepsin will be defined as the dose level immediately below the dose level at which greater than or equal to 2 patients out of 6 patients in a cohort experience a dose-limiting toxicity (DLT).

Table 3: Dose Escalation & De-Escalation Decision Rules:

Number of Patients with DLT at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level.
1 out of 3	Enter at least 3 more patients at this dose level. If 0 of these 3 additional patients experience DLT, proceed to the next dose level. If 1 or more of this group experience DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose (MAD), and the dose level below is the MTD.
$\geq$2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered).
$\leq$1 out of 6 at highest dose level below the maximally administered dose	This is generally the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

5.1.6 Dose De-escalation Decision Rules:

If a DLT is reached in the first cycle for a given patient, then the previous dose level will be utilized for that particular patient in subsequent cycles. Should that patient experience a second DLT at that lower dose, they will be removed from the study. Dose-escalation will resume until the maximally administered dose is achieved or MTD is reached.

For example, according to the schema in Table 2, if DLT is reached at dose level 1 (romidepsin 10 mg/m2 and oral 5-azacitidine 100 mg/m2), the frequency of romidepsin will be reduced to a Day 8 only dose. Dose escalation can resume starting at that point according to dose escalation rules (Table 3). The same

applies if DLT is reached at all other levels prior to dose level four.

Toxicities in cycles 2 and following may be accomodated by treating patients at one dose level below the present cohort. Toxicity in cycles 2 and beyond will not affect dose escalation for future patients.

In the Phase 2, de-escalation should follow the Phase 1 escalation guidelines. For instance, any recurrent Grade 3 toxicity, up to 3 de-escalations will be allowed, with the doses of the drugs being reduced to that described in Cohort 5 (azacytidine 300 mg Day 1-14, and romidepsin 14 mg/m2 Day 8 and 15 every 28 days)→ Cohort 4 (azacytidine 300 mg Day 1-14, and romidepsin 10 mg/m2 Day 8 and 15 every 28 days)and then Cohort 3 (azacytidine 200 mg Day 1-14, and romidepsin 10 mg/m2 Day 8 and 15 every 28 days). Any patient experienceing a Grade 4 toxicity should just be immediately dose reduced according to the guideline above. In addition, patients can be dose reduced per the treating physicians discretion.

Specifically, for hematologic toxicities, patients may receive GCSF for neutropenia. For cases where patients miss a dose secondary to a recurrent Grade 3 ANC or platelet count, or Grade 4 hematologic toxicity, dose reductions should occur as noted above at the physicians discretion (i.e. reduce to Cohort 5, then 4 then 3).

5.1.7Prophylactic Medicines and Supportive Care

Romidepsin is moderately emetogenic. Standard institutional guidelines for anti-emetics will be used. Supportive treatment may include additional anti-emetics, anti-diarrheal, anti-pyretics, anti-histamines, analgesics, antibiotics, and blood products. Patients who experience indigestion or gastroesophageal reflux symptoms on Romidepsin may be treated with Proton Pump Inhibitors (PPIs) as well as H2 blockers as clinically indicated.

Patients who have pre-existing chronic anemia prior to receiving oral 5-azacitidine and romidepsin may continue to receive erythropoietin or darbepoietin. Post cycle 1, colony stimulating growth factors (e.g., filgrastim, peg-filgrastim, and sargramostim) may be used in patients who experience hematological toxicity per American Society of Clinical Oncology (ASCO) guidelines. After initiating treatment, anemia may be managed with growth factors according to the ASCO guidelines: 2006 Update of Recommendations for the Use of White Blood Cell Growth Factors: An Evidence-Based Clinical Practice Guideline http://www.jco.org/cgi/content/full/24/19/3187. Neulasta should only be used if the interval to the next dose is ≥14 days.

Patients will be permitted to receive appropriate supportive care measures as deemed necessary by the treating physician including but not limited to the items outlined below:

- **Diarrhea:** Diarrhea should be treated promptly with appropriate supportive care, including loperamide. Loperamide should not be taken prophylactically. Patients should be instructed to begin taking loperamide at the first sign of: 1) poorly formed or loose stool, 2) occurrence of more bowel movements than usual in one day or 3) unusually high volume of stool. Loperamide should be taken in the following manner: 4 mg at first onset of diarrhea, then 2 mg after each unformed stool. The daily dose of loperamide should not exceed 16 mg/day. Loperamide should be deferred if blood or mucus is present in the stool or if diarrhea is accompanied by fever. In this setting, appropriate diagnostic microbiologic specimens should be obtained to exclude an infectious etiology. Patients should also be advised to drink liberal quantities of clear fluids.

- **Nausea/vomiting:** Romidepsin is moderately emetigenic. Ondansteron 8 mg IV will be given prior to Romidepsin injections. Nausea and vomiting should be treated aggressively, with agents such as prochlorperazine, metoclopramide, 5-HT-3 inhibitors, or benzodiazepines. Patients should be strongly encouraged to maintain liberal oral fluid intake during therapy, especially during the initial 14 days of each treatment cycle.

- **Fatigue:** May be cumulative with increasing cycles of therapy. Can be treated as indicated by the treating physician.

- **Anemia:** Treatment with oral 5-azacitidine and romidepsin can cause dose-related anemia. Transfusions or erythropoetin may be utilized as clinically indicated for the treatment of anemia, but should be clearly noted as concurrent medications.

- **Thrombocytopenia:** Treatment with Oral 5-azacitidine and Romidepsin can cause dose-related thrombocytopenia. Transfusion of platelets may be used if clinically indicated. Dose modification for thrombocytopenia is allowed in a manner consistent with the guidelines for dose modification (Section [6.3]). Prophylactic folic acid and vitamin B12 supplements may be necessary to reduce hematologic toxicity.

- **Neutropenia:** Prophylactic use of colony-stimulating factors including G-CSF, pegylated G-CSF or GM-CSF should not be utilized during the first cycle of therapy. These factors may be utilized if clinically indicated in subsequent cycles.

- **Hypokalemia or hypomagnesemia** should be corrected prior to administration of Romidepsin, and consideration should be given to monitoring potassium and magnesium in symptomatic patients (e.g. patients with nausea, vomiting, diarrhea, fluid imbalance or cardiac symptoms.)

- **Oral Mucositis:** Oral 5-azacitidine may cause mucositis (includes stomatitis or mucosal inflammation of gastrointestinal and genitourinary tracts); which may require dosage modification. Prophylactic folic acid and vitamin B_{12} supplements are necessary to reduce treatment-related mucositis.

- **Hepatotoxicity:** Liver function test abnormalities have been observed with Oral 5-azacitidine use. Persistent abnormalities may indicate hepatotoxicity and may require dosage modification.

5.1.8 Cardiac Monitoring

Minor EKG changes are not uncommon following romidepsin administration. Cardiac assessments must be performed for all study patients. **The Investigator must perform the primary assessment and is responsible for the cardiac safety of the patients (local cardiologist may be consulted, if preferred).**

5.1.8.1 Cardiac Alert Findings

In the event of an alert finding, the individual decision about a delay of administration, dose reduction, or withdrawal from the study will be made by the Investigator (in association with local cardiologist, if preferred). All alerts must be confirmed via a manual read of the patients ECG; the machine reading alone is not adequate. The following findings are considered to be cause for alert and if they occur,

should be reported as AEs or SAEs, as appropriate:

- QTc ≥500 msec
- Ventricular arrhythmia: VT (≥3 beats in a row) or VF;
- Sinus tachycardia (pulse >140/min after recumbency);
- Heart rate ≥ 120 bpm with ≥ 20 bpm increase from previous evaluation;
- New occurrence of atrial dysrhythmias (SVT, atrial fibrillation, or atrial flutter); or
- Abnormal ST and/or T-wave changes including ST depression of ≥2 mm (as measured from isoelectric line to the ST segment at a point 60 msec at the end of the QRS complex); T-wave inversion of ≥4 mm (measured from isoelectric line to peak of T-wave) as long as the main QRS vector is positive;
- Ventricular tachycardia, including Torsade de Pointes.

See Table 4 for recommended dose reductions.

5.1.8.2 Electrocardiograms

Single ECGs (12 lead) should be performed during screening (days -14 to -1) and weekly during cycle 1. Subsequently, ECG will be performed on Day 8 of each cycle to cycle 6, then at the discretion of the Investigator (within 1 hour prior to the romidepsin administration, after administration of antiemetic/premedication).

If the QTc is ≥500 msec, ECGs should be repeated 2 more times for a mean of three QTc values. Minor ECG changes are expected following romidepsin administration (refer to current Investigator's Brochure). All abnormalities must be confirmed by the investigator's manual read of the patient ECG (or local cardiologist, if preferred). Abnormal alert findings, based on manual read, should be reported as AEs or SAEs, as appropriate. ECGs must also be performed at the final study visit, whenever clinically indicated and at all visits for follow-up of AEs.

5.1.8.3 All Dosing Days

- ECGs within 1 hour prior to the administration of romidepsin (after administration of anti-emetic/pre-medication)

In case of the cardiac findings (alerts) above, an individual decision about a delay of administration, dose reduction, or withdrawal from the study will be made by the Investigator who may wish to consult with a local cardiologist.

5.2 Dose-Limiting Toxicity

A dose limiting toxicity (DLT) is defined as any toxicity not specifically included in section 5.2 that occurs within cycle 1 and is considered at least "possibly related to study drug administration." DLT is also defined as any toxicity possibly related to drug, occurring up to 7 days after completion of cycle 1 that results in a delay of initiating cycle 2. For patients who discontinue study drug administration after Cycle 1, DLT will be defined as any toxicity not specifically included in section 5.2 that occurs within 30 days of the last dose of drug on study, in cycle 1 that is considered at least possibly related to study

drug.

DLTs include:

- Hematologic dose-limiting toxicity will be defined as either Grade 4 neutropenia that does not resolve to ≤ Grade 2 within ≤ 7 days, any Grade 4 thrombocytopenia, or any Grade 5 hematologic toxicity. At least three patients in each cohort must be evaluable for hematologic toxicity.
- Non-hematologic dose-limiting toxicity will be defined as any Grade 3, 4 or 5 non-hematologic toxicity, with the specific exception of:
- Grade 3 nausea or Grade 3 vomiting that in the opinion of the investigator occurs in the setting of inadequate compliance with supportive care measures specified in Section 5.1.7 and lasts for less than 48 hours.
- Grade 3 diarrhea that in the opinion of the Investigator occurs in the setting of inadequate compliance with supportive care measures specified in Section 5.1.7 and lasts for less than 48 hours.
- Grade 3 dehydration that, in the opinion of the investigator, occurs in the setting of inadequate compliance with supportive care measures specified in Section 5.1.7 and lasts for less than 48 hours.
- Grade 3 acidosis or alkalosis that responds to medical intervention by returning to ≤ Grade 2 within 48 hours.
- Isolated (i.e. no other abnormalities) Grade 3 elevation of liver function tests (LFTs) without associated clinical symptoms, lasting for ≤ 5 days in duration.
- Isolated (i.e. no other abnormalities) Grade 3 elevation of amylase without associated clinical symptoms, lasting ≤5 days
- Grade 3 hypocalcemia, hypokalemia, hypomagnesemia, hyponatremia, or hypophosphatemia that responds to medical intervention.
- Grade 3 hypercholesterolemia.
- Grade 3 hypertriglyceridemia
- Grade 2 Alopecia
- Grade 3 Constipation
- Grade 3 Fatigue

Management and dose modifications associated with the above adverse events are outlined in Section 6.

5.3 General Concomitant Medication and Supportive Care Guidelines

Because there is a potential for interaction of oral 5-azacitidine and romidepsin with other concomitantly administered drugs through the cytochrome P450 system, the case report form must capture the concurrent use of all other drugs, over-the-counter medications, or alternative therapies. The Principal Investigator should be alerted if the patient is taking any agent known to affect or with the potential to affect selected CYP450 isoenzymes.

Carefully monitor prothrombin time (PT) and International Normalized Ratio (INR) in patients concurrently administered ISTODAX® and Coumadin derivatives

Patients must be instructed not to take any additional medications (including over-the-counter products) during the trial without prior consultation with the PI. All medications taken within 30 days of screening

and medications and supportive therapies that are administered during the study must be recorded in the patient's CRF and in the source documents. Supportive therapy, that is ongoing at baseline, will be permitted during the treatment phase of the study. If other therapy for the disease is required, continuation of the study treatment should be discussed with the lead investigator and sponsor. Concomitant medications for other medical conditions are permitted as clinically indicated subject to specific protocol requirements outlined below.

Permitted medications and treatments

- **The prophylactic use of antiemetics is strongly encouraged**, but antiemetics that significantly prolong QTc (*e.g.*, palonosetron) or that significantly inhibit CYP3A4 (*e.g.*, aprepitant) must be avoided (see Appendix 1, and 2, respectively). Granisetron and ondansetron are the antiemetics most commonly utilized in other romidepsin clinical trials.
- Patient's electrolytes (serum potassium and magnesium) must be measured, and must receive supplement infusions to correct any levels outside of those stipulated per protocol. Patients must then undergo a repeat biochemistry test to demonstrate values are within the accepted range before the patient is re-dosed with romidepsin.

Prohibited concurrent therapy

- Concomitant use of other anti-cancer therapies, including radiation, thalidomide, or other investigational agents is not permitted while subjects are receiving protocol therapy during the treatment phase of the study.
- Any medications listed in Appendix 1 which may cause QTc prolongation or inducing torsades de pointes should not be used.
- Concomitant use of CYP3A4 inhibitors with romidepsin should be avoided (excluding granisetron and ondansetron) to prevent potential increase in romidepsin exposure during concomitant treatment with these drugs. Should a patient already enrolled on this study require treatment with these drugs (as listed in Appendix 2) romidepsin must be interrupted prior to starting these drugs and should not resume until a washout period of at least 5 half-lives has elapsed.
- Strong CYP3A4 inhibitors may increase concentrations of ISTODAX® and should be avoided.
- Potent CYP3A4 inducers may decrease concentrations of ISTODAX® and should be avoided
- Any medications that have the potential to alter serum electrolytes (e.g., diuretics) should be monitored very closely for electrolyte abnormalities as these can contribute to the risk of QT prolongation and ventricular arrhythmias.

PROPHYLACTIC MEASURES

Subjects should receive allopurinol or rasburicase and other treatment considered appropriate for tumor lysis prophylaxis in cycle 1 as deemed necessary by the treating physician.

Subjects should receive prophylactic anti-emetics prior to romidepsin administration.

G-CSF may be given at each cycle. (Neulasta should only be given if there is a 14 day interval to the next dose).

As noted previously, Serum potassium (K^+) and Serum Magnesium (Mg^+) will be verified before each dose of Romidepsin. Values ≤ laboratory LLN will be corrected prior to Romidepsin administration.

5.4 Duration of Therapy

Treatment may continue until one of the following criteria applies:

- Disease progression
- Unacceptable adverse event(s), DLTs
- Withdrawal of consent
- General or specific changes in the patient's condition render the patient unacceptable for further treatment in the judgment of the investigator
- An event that in the judgment of the treating physician warrant's discontinuation of therapy
- There is no pre-specified number of cycles allowed

5.5 Duration of Follow Up

Patients will have an end of study visit 4 weeks +/- 5 days after their last dose of drug to evaluate safety. Patients will be further followed every three months after the 4-week safety evaluation for one year, or until they begin a new treatment for their disease, for evaluation of delayed toxicity. Patients removed from study for unacceptable adverse events will be followed until resolution or stabilization of the adverse event.

For patients who have stable disease or better after 6 cycles of therapy, tumor assessment will be performed by medical history, physical, CBC with differential and chemistries every 3 months until progression of disease occurs. CT (PET/CT optional) will not be routinely used to monitor complete responses after 6 months, but rather, will be used when indicated, due to concern for disease progression.

5.6 Criteria for Removal from Study

Patients will be removed from treatment when any of the criteria listed in Section 5.4 applies. The reason for study removal and the date the patient was removed must be documented in the Case Report Form.

Subjects/patients may withdraw at any time or be dropped from the study at the discretion of the investigator should any untoward effects occur. In addition, a subject/patient may be withdrawn by the investigator if he/she violates the study plan or for administrative and/or other safety reasons. The investigator or study coordinator will notify the appropriate parties within 2 business days when a subject has been discontinued/ withdrawn due to an adverse event (Section 7). When a subject discontinues/withdraws prior to study completion, all applicable activities scheduled for the final study visit should be performed at the time of discontinuation. Any adverse events present at the time of discontinuation/withdrawal will be followed until resolution.

6. DOSING DELAYS/DOSE MODIFICATIONS

6.1 Dose Delays

- Study drugs can be administered within 48 hours of their scheduled time.
- Study drugs will be held for DLTs until adverse event returns to $\leq$ Grade 2
- If interruption lasts for more than 14 days, study treatment will be discontinued.

6.2 Resuming Administration of Study Drug

For toxicities that can be treated or prevented, such as nausea, vomiting, diarrhea and neutropenia, mucositis, treatment may be resumed at the previous dose once supportive measures have been instituted and toxicity recovers to Grade 2 or less.

6.3 Dose Modifications

If oral 5-azacitidine and romidepsin administration leads to a DLT beyond cycle 1 at a given dose level, dose modifications will occur in accordance with the drugs' FDA approved package insert for romidepsin, or as outlined in the dose (de)escalation tables. These post Cycle 1 DLTs will not count towards the overall determination of MAD & MTD for this study.

6.3.1 Romidepsin Dose Modifications

Romidepsin dose modification in case of cardiac toxicity

The guidelines for cardiac monitoring and timing of ECG assessments are presented in Table 7. Prolongation of QTc ≥500 msec is considered to be an alert associated with romidepsin administration

TABLE 7: Dose Modification in Case of Cardiac Toxicity

Parameter/Symptoms	Change	Action	Dosing/ Continuation
Sinus tachycardia	Pulse >140/min after recumbency	Hold further dosing and treat appropriately. If desired, the medical monitor or a local cardiologist may be consulted.	If resolved, restart romidepsin at the next reduced level below. If not resolved, take off study.
Atrial dysrhythmia (SVT, atrial fibrillation, or atrial flutter)	New occurrence		
Prolongation of QTc compared to baseline	To ≥500 msec		
Heart rate	> 120 bpm with > 20 bpm increase from previous evaluation;		
Ventricular tachycardia	≥3 beats in a row		
Ventricular fibrillation; Torsade de Pointes	New occurrence	Hold further dosing and treat appropriately. The medical monitor should be notified immediately and local cardiologist should be consulted.	Hold further dosing until medical monitor and cardiologist evaluation is complete
A subsequent episode of any of the above, despite dose reduction		**Take off study**	
T-wave morphology	Inversion of ≥4 mm[a]	Hold further dosing and treat appropriately. If desired, the medical monitor or a local cardiologist may be consulted.	If resolved, restart romidepsin at the next reduced level below. In some patients, ST segment and T-wave morphology changes may recur despite a dose reduction. In such cases, further treatment should be held until the ECG changes resolve. If the patient experiences no concomitant clinical events, treatment may be resumed at the next reduced level below. If not resolved, take off study
ST-segment	Depression of ≥2 mm[b]		

NOTE: Cardiac findings that require dose modification should be reported as AEs or SAEs as appropriate.

[a] Measured from isoelectric line to peak of T-wave.

[b] Measured from isoelectric line to ST segment.

Romidepsin dose modification for Other Non-Hematologic and Hematologic Toxicities

All previously established or new toxicities observed any time, with the exception of those mentioned above, are to be managed as summarized in Table 8:

TABLE 8: Dose Modifications for <u>Romidepsin</u>

Dose Modifications for Romidepsin	
CTCAE Grade or Abnormal Value	Romidepsin
Thrombocytopenia	
Grade 1 (75 x 10^9/L - < LLN) *Grade 2 (50 - < 75 x 10^9/L)*	Maintain dose level *Maintain dose level*
Grade 3 or 4 thrombocytopenia: OR Thrombocytopenia that requires platelet transfusion or recurrent Grade 3 or 4 thrombocytopenia	Hold subsequent doses of therapy until thrombocytopenia returns to ≤Grade 2 or baseline and continue at full dose Hold subsequent doses of therapy until thrombocytopenia returns to ≤Grade 2 or baseline and permanently reduce dose of romidepsin to the next reduced level below.. If the same toxicity recurs despite dose reduction, patients should be taken off study.
Neutropenia (ANC)	
Grade 1 (1.5 x 10^9/L - < LLN) Grade 2 (1.0 - < 1.5 x 10^9/L)	Maintain dose level Maintain dose level
Grade 3 and 4 neutropenia:	Hold subsequent doses of therapy until specific cytopenia returns to ≤Grade 1 or baseline and continue at full dose
Febrile Neutropenia	
Febrile (≥38.5ºC) Grade 4 neutropenia or recurrent Grade 3 or 4 neutropenia:	Hold subsequent doses of therapy until neutropenia returns to ≤Grade 1 or baseline and permanently reduce dose of romidepsin to the next reduced level below.. If the same toxicity recurs despite dose reduction, patients should be taken off study
All Other Non-Hematologic Drug Toxicities (not mentioned in previous tables)	
Grade 1 or 2: Grade 3 Grade 4	Maintain dose level Hold treatment with romidepsin until toxicity returns to ≤Grade 1 or baseline, then restart therapy at full dose. If Grade 3 toxicity recurs, reduce dose of romidepsin to the next reduced level below.. This is a permanent dose reduction. Grade 4 toxicity: Hold treatment with romidepsin until toxicity returns to ≤Grade 1 or baseline, and then restart therapy at the next reduced level below.. This is a permanent dose reduction

Dose interruption or study discontinuation is not required for lymphopenia of any grade.

In general, if study drug has been held and the toxicity does not resolve, as defined above, then drug must

be discontinued.

Note: Dose reductions should not be performed for alopecia or for nausea or vomiting that was not treated with aggressive anti-emetic support.

7. ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS

7.1 Safety Evaluation Procedures

Adverse Event Reporting
An adverse event is defined as any unfavorable and unintended change in the structure, function, or chemistry of the body temporally associated with the use of the study drugs, whether or not considered related to the use of the product. Any worsening (i.e., any clinically significant adverse change in frequency and/or intensity) of a preexisting condition that is temporally associated with the use of the study drugs product, is also an adverse event.

Adverse events may occur in the course of receiving study drugs or within the follow-up period specified by the protocol, as well as from overdose (whether accidental or intentional), from abuse, and from withdrawal.

Adverse events will be graded and recorded throughout the study according to NCI-CTCAE, version 4.0. Toxicities will be characterized in terms including duration, intensity, and time to onset. Safety endpoints will include all types of adverse events, in addition to laboratory safety assessments, ECOG performance scale status, ECGs, and vital signs.

The investigator must assess all abnormal laboratory results for their clinical significance. If any abnormal laboratory result is considered clinically significant, the investigator must provide details about the action taken with respect to the test drug and about the patient's outcome.

The following safety evaluations will be performed during patient screening and at defined points during the course of the study:

- Vital signs
- Laboratory studies complete blood count (CBC), serum chemistry, urinalysis, coagulation studies (PT/PTT), pregnancy test, LDH
- Electrocardiograms (ECG)
- Physical examinations
- Performance Status Evaluation using the ECOG scale
- Adverse event monitoring using the NCI CTCAEv4.0

The STUDY FLOW CHART (Section 10) provides specific details on collection time points.

Serious Adverse Event (SAE) Definition
A serious adverse event is one that at any dose (including overdose):

- Results in death
- Is life-threatening[1]
- Requires inpatient hospitalization or prolongation of existing hospitalization

- Results in persistent or significant disability or incapacity[2]
- Is a congenital anomaly or birth defect
- Is an important medical event[3]
- Pregnancy

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

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

[3]Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in situations where none of the outcomes listed above occurred. Important medical events that may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the other outcomes listed in the definition above should also usually be considered serious. Examples of such events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse. A new diagnosis of cancer during the course of a treatment should be considered as medically important.

Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations; for example, important medical events may not be immediately life-threatening or result in death or hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above. Any adverse event is considered a serious adverse event if it is associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

Hospitalizations that do not meet these criteria are:

- reasons described in the protocol, e.g., drug administration, protocol-required testing
- social reason in the absence of an AE
- Surgery or procedure planned prior to entry into the trial

Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs (Section 7.2) and the characteristics of an observed AE (Section 7.3) will determine whether the event requires expedited (via AdEERS) in addition to routine reporting.

Ongoing monitoring of the clinical safety data in this trial will be performed consistent with procedures outlined in the DSMC charter. This review will pay particular attention to Grade 3 or 4 adverse events, serious adverse events (SAEs), adverse events that lead to discontinuation and adverse events that lead to dose reduction. Should the incidence of any particular adverse event, or combination of events, rise to a level of clinical concern the DSMC will be notified for possible review of the emergent adverse events.

7.2 Comprehensive Adverse Events and Potential Risks Lists (CAEPRs)

7.2.1 CAEPRs for Oral 5-azacitidine

7.2.1.1 Clinical Trials Experience
In Study AZA-001 and Studies 8921/9221 with SC administration of azacitidine, adverse reactions of neutropenia, thrombocytopenia, anemia, nausea, vomiting, diarrhea, constipation, and injection site erythema/reaction tended to increase in incidence with higher doses of azacitidine. Adverse reactions that tended to be more pronounced during the first 1 to 2 cycles of SC treatment compared with later cycles included thrombocytopenia, neutropenia, anemia, nausea, vomiting, injection site erythema/pain/bruising/reaction, constipation, petechiae, dizziness, anxiety, hypokalemia, and insomnia. There did not appear to be any adverse reactions that increased in frequency over the course of treatment. Overall, adverse reactions were qualitatively similar between the IV and SC studies. Adverse reactions that appeared to be specifically associated with the IV route of administration included infusion site reactions (eg, erythema or pain) and catheter site reactions (eg, infection, erythema, or hemorrhage).

In general, the most common AEs reported during the azacitidine clinical trials reflect the underlying nature of the disease and the cytotoxic properties of azacitidine. No clinically significant differences were seen when the safety data were analyzed for age, gender, or MDS subtypes.

Of the most frequently occurring adverse reactions (≥20%) in azacitidine-treated patients during the AZA-001 study, the majority was transient, non-serious, and resolved during the study.

These most common adverse reactions can be managed through delays or dose decrease of azacitidine and concomitant treatment. Further details are provided below.

Hematological Events
The most commonly reported adverse reactions associated with azacitidine treatment were hematologic events, including anemia, thrombocytopenia, neutropenia, and leukopenia. Bone marrow failure or pancytopenia were also reported less frequently. Complete blood counts should be performed at baseline, prior to administration of each dosing cycle, and whenever deemed clinically necessary to monitor potential toxicity or response. After administration of the recommended dosage for the first cycle, dosage for subsequent cycles should be adjusted. There is a greater risk of these events occurring during the first 2 cycles, after which they occur with less frequency in patients with restoration of hematological function. Most hematological adverse reactions were managed by routine monitoring of CBCs and delaying azacitidine administration in the next cycle, prophylactic antibiotics and/or growth factor support (eg, granulocyte colony stimulating factor [G-CSF]) for neutropenia and transfusions for anemia or thromobocytopenia as required.

Infections
Myelosupression may lead to neutropenia and an increased risk of infection. Serious adverse reactions such as neutropenic sepsis (0.8%) and pneumonia (2.5%) were reported in patients receiving azacitidine. Infections may be managed with the use of anti-infectives plus growth factor support (eg, G-CSF) for neutropenia.

Hemorrhagic Events
Bleeding may occur with patients receiving azacitidine. Serious adverse reactions such as GI hemorrhage (0.8%) and intracranial hemorrhage (0.5%) have been reported. Other hemorrhagic AEs have also been reported, including conjunctival hemorrhage, eye hemorrhage, and hemorrhoidal hemorrhage. Patients should be monitored for signs and symptoms of bleeding, particularly those with pre-existing or treatment-related thrombocytopenia.

Hypersensitivity
Serious hypersensitivity reactions (0.25%) have been reported in patients receiving azacitidine. In case of an anaphylactic like reaction, treatment with azacitidine should be immediately discontinued and appropriate symptomatic treatment initiated.

Injection Site Reactions
The majority of skin and SC adverse reactions were associated with the injection site. During Study AZA-001, injection site erythema and injection site reaction (nonspecific) were managed with corticosteroids and/or antihistamines, and non-steroidal anti-inflammatory drugs (NSAIDs); however, the majority of such events did not require concomitant treatment and none of these adverse reactions led to temporary or permanent discontinuation of azacitidine, or reduction of azacitidine dose. The majority of these adverse reactions occurred during the first 2 cycles and tended to decrease with subsequent cycles.

Gastrointestinal Events
Nausea and vomiting are common AEs that accompany therapy with azacitidine, especially following high doses administered rapidly by IV bolus. During the CALGB clinical trials, nonsteroidal antiemetics were used to counteract these AEs and allowed the continuation of azacitidine therapy. The majority of patients received prochlorperazine or a 5-HT3 receptor antagonist (eg, granisetron, ondansetron). During Study AZA-001, antiemetic therapy for nausea/vomiting most commonly included metoclopramide, ondansetron, or domperidone. Constipation has also been commonly reported during treatment with azacitidine and may be a consequence of the recommended antiemetic pre-treatment. During Study AZA-001, if treated, constipation was most commonly managed with stool softeners and/or laxatives (eg, lactulose and coloxyl with senna).

Diarrhea is also a commonly reported adverse event associated with azacitidine therapy. Diarrhea may be more frequent and/or more severe in patients receiving orally administered azaciditine. It is recommended that patients experiencing diarrhea associated with azacitidine be managed according to the publication in the Journal of Clinical Oncology (Benson, 2004). A dose reduction of oral azacitidine may be appropriate based on the severity of diarrhea observed and the response to treatment intervention.

Renal Adverse Reactions
Severe renal tubular dysfunction has been reported in patients receiving azacitidine. This event may be manifested as renal tubular acidosis (serum bicarbonate < 20 mEq/L in association with alkaline urine), hypophosphatemia, hypokalemia, or hyponatremia, with or without increases in serum creatinine and BUN. Serum bicarbonate, BUN, and creatinine should be monitored periodically and dosage adjustments may need to be made.

Hepatic Adverse Reactions
Patients with extensive tumor burden due to metastatic disease have been rarely reported to experience progressive hepatic coma and death during azacitidine treatment.

7.2.1.2 Serious Adverse Reactions
Through 18 May 2012, approximately 5,300 patients have been exposed to azacitidine during completed or ongoing studies including 1,120 in Celgene-sponsored studies and 4,190 in non-Celgene-sponsored studies.

The most frequently reported adverse reactions with azacitidine treatment were hematological reactions including anemia, thrombocytopenia, neutropenia and leukopenia, gastrointestinal events including nausea, vomiting, abdominal pain, constipation, diarrhea, and injection site reactions (with SC administration). Adverse reactions associated with intravenously administered azacitidine were similar in frequency and severity compared with subcutaneously-administered azacitidine. The overall safety profile of azacitidine from the ongoing clinical studies is consistent with that described in this IB; however, diarrhea may be more frequent and/or more severe in patients receiving orally-administered azacitidine.

The most common serious adverse reactions noted from the pivotal study and also reported in the supporting studies included febrile neutropenia and anemia. Other reported serious adverse reactions included neutropenic sepsis, pneumonia, thrombocytopenia, and hemorrhagic events (eg, cerebral hemorrhage).

Post-marketing Safety Data
Adverse drug reactions reported from post-marketing experience have been similar in type and severity to those reported in azacitidine clinical trials.

7.2.2 CAEPRs for Romidepsin

Toxicities included nausea, vomiting, fatigue, and transient thrombocytopenia and granulocytopenia[4].

7.2.2.1 Clinical Trials Experience

In a study with 71 patients, the median number of administered cycles was four (1-to 72 cycles). Among the administered doses 76% were full doses, 7% were escalated doses, and 17% were reduced doses. Eight doses were held; three doses in three patients were held as a result of thrombocytopenia (< 50 x 10^9/L), two were held for persistent grade 3 nausea, and three were held as a result of persistent grade 3 fatigue[4].

Common nonhematologic adverse effects (any grade) included fatigue (41%), nausea (52%), vomiting (20%), and anorexia (21%). Hematologic abnormalities included leukopenia (31%), granulocytopenia (37%), lymphopenia (21%), thrombocytopenia (39%), and anemia (37%). Transient elevations of liver function tests, AST or ALT, were observed in 13 patients; two additional patients had isolated grade 1 hyperbilirubinemia. Hyperuricemia was noted in 11 patients (eight patients with grade 1 and three patients with grade 3), and hypophosphatemia was noted in six different patients. ECG changes were noted consisting of asymptomatic T-wave flattening (71%) or ST segment depression (9%). Toxicities in later cycles mirrored those observed in the first cycle. Infections occurred in 38 patients (54%) over 58 cycles (11%), including bacterial infections of the skin and upper respiratory, pulmonary, GI, and urinary tracts; bacteremia; and sepsis, and were not related to neutropenia[4].

The most common AEs reported among subjects who received romidepsin monotherapy include GI disturbances (nausea, vomiting, constipation, diarrhea), hematologic toxicities (anemia, thrombocytopenia, neutropenia), and asthenic conditions (fatigue, asthenia, lethargy). Other types of events commonly seen with romidepsin include anorexia, clinical chemistry abnormalities (hypocalcemia, hypoalbuminemia, hyperglycemia, and hypomagnesemia), pyrexia, and taste disturbances.

Overall, the incidence of AEs was higher among those with hematologic malignancies, including T-cell lymphomas (343 of 353 subjects; 97%) than among those with solid tumors (337 of 428 subjects; 79%). Review of AEs by system complex showed that particular types of AEs generally occurred at a higher incidence among subjects with hematologic malignancies than those with solid tumors, including gastrointestinal disorders (77% versus 61%, respectively), general disorders and administration site conditions (74% versus 57%, respectively), blood and lymphatic system disorders (43% versus 24%, respectively), infections and infestations (39% versus 13%, respectively), and skin and subcutaneous tissue disorders (29% versus 13%, respectively). Although the incidence of AEs was higher among subjects with hematologic malignancies than in subjects with solid tumors, the particular types of AEs reported were generally similar by indication.

Overall, the incidence of Grade 3 or greater AEs among subjects who received romidepsin monotherapy was higher among these with hematologic malignancies (227 of 353 subjects; 64%) than among those with solid tumors (235 of 428 subjects; 55%). As was the case for AEs overall, the particular types of Grade 3 or higher AEs reported were generally similar by indication.

7.2.2.2 Summary of SAEs

The incidence of SAEs, regardless of relationship to romidepsin, among subjects receiving romidepsin monotherapy was similar among those with hematologic malignancies and solid tumors (37% versus 34%, respectively). Furthermore, the SAE profile was generally similar between subjects with hematologic malignancies and solid tumors. Among the 353 subjects receiving romidepsin monotherapy with hematologic malignancies, the most commonly reported SAEs included pyrexia (21 subjects; 6%), neutropenia (including the MedDRA preferred terms (PT) neutrophil count decreased and neutrophil count) (16 subjects; 5%), thrombocytopenia (including the MedDRA PT (s) platelet count decreased and platelet count) (16 subjects; 5%), hypotension NOS (12 subjects; 3%), and dehydration and febrile neutropenia (11 subjects each; 3%). Among the 428 subjects receiving romidepsin monotherapy with solid tumors, the most commonly reported SAEs were vomiting NOS (26 subjects; 6%), nausea (24 subjects; -6%), dyspnea (17 subjects; 4%), anemia (including the MedDRA PT(s) hemoglobin decreased and hemoglobin) (15 subjects; 4%), and dehydration and fatigue (12 subjects each; 3%).

Three deaths occurred among patients with CTCL while on study, and three deaths occurred within 30 days of removal from study. Those who died on study one had hypertrophic cardiac disease with significant valvular pathology but no evidence of acute infarction or myocyte injury. Two patients died from sepsis 10 and 12 days after administration of romidepsin, one patient with *Escherichia coli* and another with methicillin-resistant *Staphylococcus aureus*. Each of the three patients who died within 30 days of study removal had been removed as a result of progression of disease and died after receiving cytotoxic chemotherapy[4].

Additional data on the most commonly reported SAEs is available in the investigator brochure.

7.2.2.3 Cardiovascular Effects

There have been reports of cardiovascular effects of HDAC inhibitors including an effect on QTc prolongation[18,19,20] Data on File with Celgene Corporation 2006. Several treatment-emergent morphological changes in ECGs (including T wave and ST-segment changes) have been reported in clinical studies with romidepsin. Many of these ECG morphologic abnormalities were determined by automated machine readings and were also observed at baseline. These ECG changes were transient and were not associated with functional cardiovascular changes or with symptoms. No cardiac events of torsade de pointes have been reported. A comprehensive evaluation of the cardiac effects of romidepsin, including rigorous statistical analyses that followed the guidelines specified in International Conference

on Harmonisation (ICH) E14, was performed under the direction of an expert cardiologist. Analyses performed were primarily based on data from 3 clinical studies of romidepsin: Study GPI-04-0001 in subjects with CTCL; NCI Study 1312 in subjects with CTCL, PTCL, or other T-cell lymphomas; and Study GPI-06-0005 in subjects with advanced solid tumors or hematologic malignancies.

A series of analyses were performed on the pool of studies as well as on individual study data to assess the impact of romidepsin on QTc prolongation, and, for Studies GPI-04-0001 and GPI-06-0005, to determine the potential confounding impact on this parameter of antiemetics given prior to romidepsin. In an analysis of the romidepsin concentration-QTc relationship based on data from 110 subjects in 3 clinical studies, a mean 2.7 msec increase (90% confidence interval [CI] upper bound 5.3 msec) in QTcF interval was measured following infusion of romidepsin when compared to baseline post-antiemetic administration. The reported increase in QTcF interval was a mean 5 msec (90% CI upper bound 7.7 msec) in a subanalysis from Studies GPI-04-0001 and GPI-06-0005 (n=74), comparing QTc interval pre-antiemetic administration to post-infusion. Data from NCI Study 1312 shows that this QTcF change persists to 24 hours, with a return to baseline by 48 hours, while in the smaller GPI-06-0005 study, QTcF peaked 2 hours post-infusion and there was no QTcF effect seen at 24 hours. Based on these analyses, it was concluded that a significant portion of the change in QTcF was associated with anti-emetic administration.

In all studies, mean HR was shown to increase by approximately 10 bpm after study drug administration, with a return to baseline by 24 hours. This rise in HR and the resulting deficiencies in standard QT heart rate correction formulae likely contribute to an artifactual calculation in the change in the QTc interval when compared to baseline. Pharmacodynamic models, where the predominant effect seen was related to HR, with no evidence of a concentration – QTc relationship, support this hypothesis.

Shift tables showed that, in this disease population, evidence of morphologic ECG abnormalities existed at baseline. In addition, the frequency of treatment emergent abnormalities was similar to the frequency of abnormal to normal transitions, particularly between dosing days. ECG changes were not associated with functional impairment.

Per current ICH E14 guidelines, the findings from these studies show that the QTc changes are below the regulatory threshold of concern, particularly considering that these studies were conducted in a target population of generally older subjects with advanced malignancies, often with significant comorbidities and concomitant medication usage. Notably, there were no absolute QTc values greater than 480 msec, no increases of ≥ 60 msec and no cardiac events of torsade de pointes. One subject had a ventricular arrhythmic event, but also had evidence of intracardiac lymphoma. Therefore, based upon these data, romidepsin does not have a significant effect on the QT interval. There is a mild effect on HR that is no longer apparent 20 hours after study drug administration. Due to this effect on HR, there may be an artifactual change in the QTc interval due to the inherent inadequacy of correction formulae as the HR moves away from 60 bpm. In addition, there does appear to be a transient change in the T-wave / ST-segment in some subjects that may affect the precise determination of the T-wave in a clinical setting.

After the data cutoff date for this protocol introduction, a single report of QTc prolongation to a maximum value of 534 msec, from a minimum baseline value of 437 msec, has been reported from a subject in a clinical study of romidepsin and bortezomib for multiple myeloma. The event was reported as possibly related to romidepsin and unlikely related to bortezomib. The subject had also received ondansetron, which has been reported to prolong QTc.

7.2.2.4 Efficacy in Humans

To date more than 870 subjects have received romidepsin in clinical trials. Antitumor activity has been observed in both solid and hematologic tumor types.

CTCL

The activity of romidepsin was evaluated in a total of 135 evaluable subjects (EP) with CTCL in Study GPI-04-0001 and NCI Study 1312. Across all 135 evaluable subjects with CTCL, the overall response rate (ORR) was 41% (55/135) and the complete response (CR) rate was 7% (10/135). Subjects with advanced disease had a similar ORR as subjects with earlier stage disease: 42% for ≥Stage IIB and 38% for Stage I or IIA disease. Romidepsin was active in all sites of disease, including skin, lymph nodes, viscera, and blood. The median duration of response was 454 days (14.9 months). Although the median time to response was 57 days (1.9 months), in some cases an objective response to romidepsin was achieved after ≥6 months. Across all 135 subjects included in the pooled EP Analysis Set, median time to disease progression was 252 days (8.3 months). In Study GPI-04-0001, treatment alleviated pruritus in most subjects (48 of 52, 92%) who entered the study with this symptom.

PTCL and other T-cell Lymphomas

The activity of romidepsin was evaluated in a Phase 2, NCI-sponsored study in 48 subjects with PTCL or other T-cell lymphomas. Of these 48 subjects, 28 had PTCL and 20 had other T-cell lymphomas, including angioimmunoblastic, primary cutaneous large T-cell, gamma-delta T-cell, and anaplastic large T-cell lymphomas[17]**.**

Among all 48 subjects, the ORR (CR+PR) was 31% (15/48). The CR and PR rates were 8% (4/48) and 23% (11/48), respectively. Fifteen percent (15%; 7/48) of subjects experienced stable disease. When response was evaluated among the 34 subjects who received ≥2 cycles of therapy, the ORR was 44% (15/34) and the CR and PR rates were 12% (4/34) and 32% (7/34), respectively. Twenty-one percent (7/34) of subjects who received ≥2 cycles of therapy experienced stable disease.

Solid Tumors

The activity of romidepsin in combination with gemcitabine has been evaluated in a Phase 1, study in 33 subjects with pancreatic cancer and other solid tumors (breast, lung, ovarian, and other). Overall, 45% (15/33) experienced stable disease. Of these 15 subjects, 12 experienced stable disease for >4 cycles, including 5 subjects with pancreatic cancer, 4 with breast cancer, and 1 each with NHL and ovarian and ampullary cancer.

Complete and updated adverse events are available in the Investigational Drug Brochure and the IND Safety Letters.

7.2.2.5 Discontinuations

Sixteen patients developed progression of disease; seven patients withdrew from study, mainly to seek alternative therapy; two patients withdrew as a result of adverse events of infection and fatigue, and three patient died on study (discussed earlier). Progression of disease without evidence of response was noted in fifteen patients [4].

7.2.2.6 Dose Modifications

Protocol-mandated dose reductions were required for 42 doses in 20 patients for the following reasons: 33 dose reductions were a result of thrombocytopenia (> 50 but < 75 x 10^9/L), four were a result of granulocytopenia (> 0.5 but < 1 x 10^9 cells/L), three were a result of persistent nausea, and two were a result of fatigue. The remainder of the doses less than 14 mg/m^2 (n = 208) were administered as permanent dose reductions in patients who previously had a dose held or had one or more protocol-mandated dose reductions [4].

7.3 Adverse Event Characteristics

- **CTCAE term (AE description) and grade:** The descriptions and grading scales found in the revised NCI Common Terminology Criteria for Adverse Events (CTCAE) version 4.0 will be utilized for AE reporting. A copy of the CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov).

- **'Expectedness'**: AEs can be 'Unexpected' or 'Expected' (see Section 7.2 above) for expedited reporting purposes only.

- **Attribution** of the AE:
- Definite – The AE *is clearly related* to the study treatment.
- Probable – The AE *is likely related* to the study treatment.
- Possible – The AE *may be related* to the study treatment.
- Unlikely – The AE *is doubtfully related* to the study treatment.
- Unrelated – The AE *is clearly NOT related* to the study treatment.

7.4 Expedited Adverse Event Reporting

The Principal Investigator agrees to provide appropriate parties with copies of all serious adverse experiences*, within two working days. Additionally, the Principal Investigator agrees to report any pregnancy occurring in association with use of a oral 5-azacitidine and romidepsin to the appropriate parties.

Expedited reporting by investigator to Celgene
Serious adverse events (SAE) are defined above. The investigator must inform Celgene in writing using a Celgene SAE form or MEDWATCH 3500A form of any SAE within 24 hours of being aware of the event. The written report must be completed and supplied to Celgene by facsimile within 24 hours/1 business day. The initial report must be as complete as possible, including an assessment of the causal relationship between the event and the investigational product(s), if available. Information not available at the time of the initial report (e.g., an end date for the adverse event or laboratory values received after the report) must be documented on a follow-up report. A final report to document resolution of the SAE is required. The Celgene tracking number (RM-NHL-PI-0010) and the institutional protocol number should be included on SAE reports (or on the fax cover letter) sent to Celgene. A copy of the fax transmission confirmation of the SAE report to Celgene should be attached to the SAE and retained with the patient records.

Celgene Drug Safety Contact Information:
Celgene Corporation
Global Drug Safety and Risk Management
Connell Corporate Park
300 Connell Dr. Suite 6000
Berkeley Heights, NJ 07922
Fax: (908) 673-9115
E-mail: drugsafety@celgene.com

Report of Adverse Events to the Institutional Review Board
Reportable information should always be reported by the PI directly to the IRB within 5 working days from when the PI learns of the event or new information.

Investigator Reporting to the FDA
The investigator is responsible for reporting any SAEs to the FDA. **Serious** adverse events (SAEs) that are **unlisted/unexpected, and at least possibly associated to the drug,** and that have not previously been reported in the Investigators brochure, or reference safety information document should be reported promptly to the Food and Drug Administration (FDA) by telephone (1-800-332-1088), fax (1-800-FDA-0178), or via MedWatch Online. Fatal or life threatening SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 7 calendar days after awareness of the event. All other SAEs that meet the criteria for reporting to the FDA must be reported to the FDA within 15 calendar days after awareness of the event. A clear description of the suspected reaction should be provided along with an assessment as to whether the event is drug or disease related.

Adverse event updates/IND safety reports
Celgene shall notify the Investigator via an IND Safety Report of the following information:

- *Any AE associated with the use of drug in this study or in other studies that is both serious and unexpected.*
- *Any finding from tests in laboratory animals that suggests a significant risk for human subjects including reports of mutagenicity, teratogenicity, or carcinogenicity.*

The Investigator shall notify his/her IRB/EC promptly of these new serious and unexpected AE(s) or significant risks to subjects.

The Investigator must keep copies of all AE information, including correspondence with Celgene and the IRB/EC, on file (see Section 11.4 for records retention information).

7.4.1 Expedited Reporting Guidelines

Note: All deaths on study require expedited reporting regardless of causality. Attribution to treatment or other cause must be provided.

- Expedited AE reporting timelines defined:
  - "1 business day; 5 calendar days" – The investigator must initially report the AE within 1 business day of learning of the event followed by a complete report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete report on the AE must be submitted within 10 calendar days of the investigator learning of the event.

- Any medical event equivalent to CTCAE grade 3, 4, or 5 that precipitates hospitalization (or prolongation of existing hospitalization) must be reported regardless of attribution and designation as expected or unexpected with the exception of any events identified as protocol-specific expedited adverse event reporting exclusions.

- Any event that results in persistent or significant disabilities/incapacities, congenital anomalies, or birth defects must be reported if the event occurs following treatment.

7.4.2 Protocol-Specific Expedited Adverse Event Reporting Exclusions

For this protocol, certain AEs/grades are exceptions to the Expedited Reporting Guidelines and do not require expedited reporting. The following AEs must be reported through the routine reporting mechanism (Section 7.4):

Table 8: CTCAE AE Reporting Exclusions

CTCAE Category	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution
Blood/Bone Marrow	Neutropenia <7 days without fever	4	No	Yes
Blood/ Bone Marrow	Thrombocyotopenia <7 days without bleeding	4	No	Yes

7.5 Pregnancy on Study

Both Oral 5-azacitidine and Romidepsin can cause fetal harm when administered to a pregnant woman. There are no adequate and well-controlled studies of either agent in pregnant women.

Women of child bearing potential will be advised to avoid becoming pregnant while receiving treatment with either Oral 5-azacitidine, Romidepsin or the combination . Women enrolled in this study should either be post-menopausal, free from menses for > 2 years, surgically sterilized, or willing to use 2 adequate barrier methods of contraception to prevent pregnancy or agree to abstain from heterosexual activity throughout the study, starting with visit 1. Women of child-bearing potential must have a negative serum pregnancy test (β-hCG) within 72 hours prior to receiving the first dose of romidepsin. Men enrolled in the study must also agree to an adequate method of contraception for the duration of the study.
If a patient or their partner inadvertently becomes pregnant while on study, the patient will immediately be removed from the study and the drugs will be discontinued. Investigators will follow the patient monthly and document the patient's status until the pregnancy has been completed or terminated. The outcome of the pregnancy will be reported to Appropriate parties within 24 hours if the outcome is a serious adverse event (i.e. death, abortion, congenital anomaly, or other disabling or life-threatening complication to the mother or newborn). If a male patient's partner becomes pregnant on study, the pregnancy will be reported to appropriate parties. Reporting of pregnancy will follow the guidelines outlined in Section 7.4.

Pregnancies

Pregnancy of a female subject or the female partner of a male subject occurring while the subject is on romidepsin or within 4 weeks after the subject's last dose of romidepsin are considered expedited

reportable events. If the subject is on romidepsin, it is to be discontinued immediately. The pregnancy must be reported to Celgene Drug Safety within 24 hours of the Investigator's knowledge of the pregnancy by phone and facsimile using the SAE Form.

The Investigator will follow the pregnant female until completion of the pregnancy, and must notify Celgene Drug Safety of the outcome as specified below. The Investigator will provide this information as a follow-up to the initial SAE.

If the outcome of the pregnancy meets the criteria for immediate classification as a SAE (i.e., spontaneous abortion [any congenital anomaly detected in an aborted fetus is to be documented], stillbirth, neonatal death, or congenital anomaly), the Investigator should follow the procedures for Expedited Reporting of SAEs to Celgene (i.e., report the event to Celgene Drug Safety by facsimile within 24 hours of the Investigator's knowledge of the event).

In the case of a live "normal" birth, Celgene Drug Safety should be advised as soon as the information is available.

7.6 Routine Adverse Event Reporting

All Adverse Events **must** be reported in routine study data submissions.

For 30 days subsequent to study completion or withdrawal, new onset adverse events will be captured. Follow up of these events will follow the same procedure as described above for AEs observed during the study period.

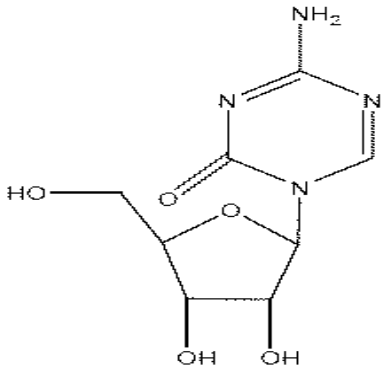
8. PHARMACEUTICAL INFORMATION

A list of the adverse events and potential risks associated with the investigational or commercial agents administered in this study can be found in Section 7.2.

8.1 Oral 5-azacitidine

Product description:
Vidaz® (5-AZA for injectable suspension) contains AZA, which is a pyrimidine nucleoside analog of cytidine. It has the following chemical structure:

Chemical Structure of Azacitidine



Azacitidine is 4-amino-1-â-D-ribofuranosyl-s-triazin-2(1H)-one. The empirical formula is C8H12N4O5. The molecular weight is 244.

Azacitidine is a white to off-white solid. Azacitidine was found to be insoluble in acetone, ethanol, and methyl ethyl ketone; slightly soluble in ethanol/water (50/50), propylene glycol, and polyethylene glycol; sparingly soluble in water, water saturated octanol, 5% dextrose in water, N-methyl-2-pyrrolidone, normal saline and 5% Tween 80 in water; and soluble in dimethylsulfoxide (DMSO).

The finished product is supplied in a sterile form for reconstitution as a suspension for subcutaneous injection or reconstitution as a solution with further dilution for intravenous infusion. Vials of VIDAZA® contain 100 mg of azacitidine and 100 mg mannitol as a sterile lyophilized powder.

Solution preparation:
Reconstitute the number of azacitidine vials to achieve the desired dose. Reconstitute each vial with 10 mL sterile water for injection. Vigorously shake or roll the vial until all solids are dissolved. The resulting solution will contain azacitidine 10 mg/ml. The solution should be clear. Parenteral drug product should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit.
Withdraw the required amount of azacitidine solution to deliver the desired dose and inject into a 50-100 mL infusion bag of either 0.9% Sodium Chloride Injection or Lactated Ringer's Injection. Unused portions should be properly discarded.

Intravenous Solution Incompatibility
Azacitidine is incompatible with 5% dextrose solutions, Hespan or solutions that contain bicarbonate. These solutions have the potential to increase the rate of degradation of azacitdine and should be avoided.

Storage requirements:
Store unreconstituted vials at 25º C (77º F); excursions permitted to 15º-30º C (59º-86º F) (See USP Controlled Room Temperature). There is no need to protect azacitidine from exposure to light.

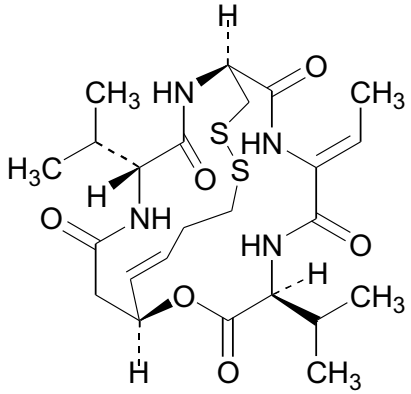
Stability:
Azacitidine reconstituted for intravenous administration may be stored at 25º C (77 º F), but administration must be completed within 1 hour of reconstitution. Unused portions should be properly discarded.

Administration
Azacitadine solution should be administered intravenously. Administer the total dose over a period of 10-40 minutes. The administration must be completed with 1 hour of reconstitution of the azacitidine vial. Unused portions should be properly discarded.

8.2 Romidepsin

Romidepsin is a unique bicyclic depsipeptide originally isolated from *Chromobacterium violaceum* strain 968.[7] Romidepsin is an antineoplastic agent that has been identified as a novel HDAC inhibitor. Romidepsin has been shown to induce hyperacetylation of histones and other nonhistone protein species resulting in a variety of phenotypic changes, induction of the upregulation of gene transcription, G1 and G2/M arrest of the cell cycle, morphological reversion of transformed cells, cell growth inhibition, apoptotic cell death, and inhibition of angiogenesis.

Figure 2: Chemical Structure



The molecular formula of romidepsin is $C_{24}H_{36}N_4O_6S_2$, its molecular weight is 540.71, and its chemical name is: (1S,4S,7Z,10S,16E,21R)-7-ethylidene-4,21-bis(1-methyletheyl)-2-oxa-12,13-dithia-5,8,20,23-tetraazabicyclo[8.7.6]tricos-16-ene-3,6,9,19,22-pentone.

Mechanism of Action

Romidepsin is a unique bicyclic depsipeptide originally isolated from Chromobacterium violaceum strain 968.[7] Results of early nonclinical studies showed that romidepsin inhibited the growth of the Ha-ras-transformed NIH3T3 clonal cell line, Ras-1, and induced reversion of the transformed morphology to normal within 1 day at a concentration of 2.5 ng/mL.[8] While mRNA expression of the c myc oncogene in Ras-l cells was decreased in the presence of romidepsin, Ha-ras mRNA expression was unaffected by 24-hour exposure to 2.5 ng/mL of romidepsin. Romidepsin blocked cell cycle transition from G0/G1 to S phase and induced nuclear quiescence. The course of c-myc suppression paralleled that of G0/G1 arrest and correlated with the morphologic reversion of the transformed cells. These results led to the proposal that the growth inhibition and G0/G1 arrest resulted from romidepsin blocking the ras-mediated signal transduction pathway.[9] Other investigations of the effect of romidepsin on G1 to S transition of the cell cycle showed that romidepsin inhibits signal transduction through MAP kinase and causes p53-independent G1 arrest.[10] Romidepsin has also been identified as a histone deacetylase (HDAC) inhibitor similar to trichostatin A based on its ability to cause arrest of the cell cycle at both G1 and G2/M phases, to induce internucleosomal breakdown of chromatin, and to inhibit intracellular HDAC activity resulting in an accumulation of marked amounts of acetylated histone species within M 8 cells.[11]

In both *in vitro* and *in vivo* systems, romidepsin has been shown to elicit a range of biological activities, including HDAC inhibition, induction or repression of gene expression, cell cycle arrest, differentiation, cell growth inhibition, apoptotic cell death, morphological reversion of transformed cells, and inhibition of angiogenesis.[15] The manner in which romidepsin and other HDAC inhibitors exert their biological effects has not been fully elucidated. The current view is that these agents inhibit, to a greater or lesser extent, the activity of Class I (HDACs 1, 2, 3, 8), Class II (HDACs 4, 5, 6, 7, 9, 10), and Class IV (HDAC 11) HDACs, causing chromatin remodeling and altered gene expression, which results in biological effects that are deleterious to tumor cell growth and survival. There is a growing body of evidence that HDAC inhibitors can also target substrates other than histones and that the posttranslational modification of cellular proteins by acetylation may play an important role in the biological activities of HDAC inhibitors.[16]

Product description:

ISTODAX® (Romidepsin) is a histone deacetylase (HDAC) inhibitor indicated for the treatment of cutaneous T-cell lymphoma (CTCL) in patients who have received at least one prior systemic therapy.

Solution preparation:
Romidepsin is supplied as a kit including a sterile, lyophilized powder in a single-use vial containing 10 mg of romidepsin and 20 mg of the bulking agent, povidone, USP. In addition, each kit includes one sterile Diluent vial containing 2 mL (deliverable volume) of 80% propylene glycol, USP, and 20% dehydrated alcohol, USP.

NDC 46026-983-01: ISTODAX® KIT containing 1 vial of romidepsin, 10 mg and 1 vial of diluent for romidepsin, 2 mL per carton

Storage requirements:

The dual pack is to be stored at 20 to 25ºC (68 to 77°F), excursions permitted between 15 to 30°C (59 to 86°F) [USP controlled room temperature].Romidepsin (for infusion) is stable for at least 36 months at 25°C/60% relative humidity (RH) as well as for 6 months at 40°C/75% RH and is stable against heat (for 3 months at 50°C) and humidity (for 3 months at 25°C/83% RH). The drug should be reconstituted by appropriately trained personnel using aseptic technique. A volume of 2 mL of reconstitution diluent is added to the lyophilized powder and swirled until contents of the vial are free from visible particles. The reconstituted product stock solution at 5 mg/mL is chemically stable for at least 8 hours at room temperature. However, whenever possible, drug should be prepared within 4 hours of dose administration. A volume of the 5 mg/mL stock solution containing the appropriate dose for the patient will be diluted in 0.9% Sodium Chloride Injection, USP (0.9% saline) for intravenous infusion, as directed by the protocol. This dilution should result in a final drug concentration within the demonstrated stability range of 0.02 to 0.16 mg/mL for reconstituted romidepsin, that is compatible with polyvinyl chloride (PVC), ethylene vinyl acetate (EVA), and polyethylene (PE) intravenous infusion bags; glass bottles may also be used. The romidepsin infusion solution is chemically stable for at least 24 hours at room temperature. However, whenever possible, drug should be prepared within 4 hours of dose administration.

Administration
Romidepsin should be handled in a manner consistent with recommended safe procedures for handling cytotoxic drugs.

The lyophilized, sterile finished product contains romidepsin, 10 mg/vial and 20 mg/single use vial Povidone, USP, and hydrochloric acid to adjust pH. Romidepsin (for infusion) is supplied in a dual-pack configuration with a single use Diluent for Romidepsin vial that contains 2 mL of 80% Propylene Glycol, USP, and 20% Dehydrated Alcohol (ethanol), USP; sterile for use in reconstitution of Romidepsin (for Infusion).

Romidepsin must be reconstituted with the supplied diluent and further diluted with 0.9% Sodium Chloride Injection, USP before intravenous infusion.

- Each 10 mg single-use vial of romidepsin must be reconstituted with 2 mL of the supplied Diluent. With a suitable syringe, aseptically withdraw 2 mL from the supplied Diluent vial, and slowly inject it into the romidepsin for injection vial. Swirl the contents of the vial until there are no visible particles in the resulting solution. The reconstituted solution will contain the romidepsin 5 mg/mL. The reconstituted the romidepsin solution is chemically stable for at least 8 hours at room temperature.
- Extract the appropriate amount of the romidepsin from the vials to deliver the desired dose, using proper aseptic technique. Before intravenous infusion, further dilute the romidepsin in 500 mL 0.9% Sodium Chloride Injection, USP.

• Infuse over 4 hours.

The diluted solution is compatible with polyvinyl chloride (PVC), ethylene vinyl acetate (EVA), polyethylene (PE) infusion bags as well as glass bottles, and is chemically stable for at least 24 hours when stored at room temperature. However, it should be administered as soon after dilution as possible.

Please refer to the FDA approved package insert for further information[27].

Supplier(s)

Celgene Corporation will supply ISTODAX® (romidepsin) to study participants at no charge.

Allos Therapeutics will supply Oral 5-azacitidine to study participants at no charge

3.3.3 Non-Clinical Studies

Potent antitumor effects of romidepsin have been demonstrated both in vitro and in vivo.[7,12] In vitro, romidepsin exerted antiproliferative activity against 12 human solid tumor cell lines (IC50 ranged from 0.5 to 5.9 nM), but was less potent against cultured normal cells. It was found that the longer the duration of romidepsin exposure, the lower the concentration of drug necessary to induce the antiproliferative activity. Similar IC50 values were found in a study of 13 lymphoid cell lines.[15] In a severe combined immunodeficiency disease (SCID) mouse lymphoma model, male mice inoculated intraperitoneally (IP) with U-937 cells and treated with romidepsin (0.1 to 1 mg/kg, IP) once or twice a week survived longer [median survival times of 30.5 days (0.56 mg/kg) and 33 days (0.32 mg/kg)], than saline-treated mice (20 days).[14] Two of 12 mice treated with 0.56 mg/kg romidepsin survived past the observation period of 60 days.

Romidepsin Monotherapy

As of 31 December 2008, a total of 781 subjects received at least one dose of romidepsin as monotherapy, including 353 subjects with hematologic malignancies and 428 subjects with solid tumors. The population of 428 subjects with solid tumors includes 26 pediatric subjects treated with romidepsin in NCI Study ADVL0212.

8.3 Agent Accountability

Agent Inventory Records – The investigator, or a responsible party designated by the investigator, will maintain a careful record of the inventory and disposition of all agents received from appropriate parties.

9. Pharmacokinetic & Pharmacodynamic Studies

9.1 Pharmacokinetic Studies

The pharmacokinetic studies are noted on Figure 1.

9.2 Pharmacodynamic Studies

The pharmacodynamic studies are noted on Figure 1.

9.2.1Biopsies (Optional)

The patient will have the option of checking off boxes within the main consent form for this study to indicate consent or denial for the following optional studies.

A lymph node fine needle aspiration (FNA), core-needle biopsy or bone marrow biopsy will be performed prior to therapy and on between day 8 and 21 of cycle 1 after oral 5-azacitidine and romidepsin administration. Fine needle aspirations will be performed as follows:

Fine Needle Aspirations (FNA): A series of FNAs will be performed on palpable tumors in consenting patients as a function of drug exposure.

In general, at least 6 aspirations will be made using a sterile 10 cc syringe over the larger volume of the palpable tumor. Once the needle is placed into the tumor, the plunger will be withdrawn and pushed several times to obtain a sufficient sample from that location. Once withdrawn from the tumor, the syringe will be flushed clean in 1-2 mL of RPMI. This procedure will be repeated up to 6 times in one tumor, flushing the syringe needle in the same 1-2 mL of RPMI. Previous experiences in mice have established that performing fewer than 6 aspirations on any given tumor is associated with irreproducible results. Once the samples are acquired, the cells will be centrifuged, and resuspended in 1 mL of RPMI.

Correlations with the results of these studies will then be compared to the pharmacokinetic profile in order to establish the relationship with concentration, and the previous data obtained on the in vitro studies.

Tumor Tissue Biopsies: The biopsies will be performed by a surgeon if necessary, or by interventional radiology. A cytopathologist will evaluate the biopsy for tumor cells microscopically at the time of the biopsy. If multiple lymph nodes are available, the safest lymph node will be selected. The choice of FNA versus biopsy will be made based on safety, accessibility, and likelihood of being pathologic. A biopsy confirming recurrent or refractory disease is mandatory for participation in study. The second FNA or biopsy is encouraged, but is not mandatory. Correlative studies will be performed on de-identified tissue samples. Tissue will be labeled only with the protocol-specific unique identifier.

Bone Marrow Biopsy: a bone marrow aspirate and biopsy will be performed by the study team.

9.2.2 Handling of Specimens

A frozen section will be performed to determine if there is lymphoma. Thereafter, biopsies may be snap-frozen or sent for tissue processing, and flagged for use in the protocol. Paraffin blocks will be made to confirm histological diagnosis, and appropriate immunohistochemical staining will be performed.

Remaining tissue will be used for the pharmacodynamic analysis.

10. STUDY CALENDAR

Baseline evaluations are to be conducted as indicated in the table below, prior to start of protocol therapy. Scans must be done $\leq$4 weeks prior to the start of therapy. In the event that the patient's condition is deteriorating, laboratory evaluations should be repeated within 48 hours prior to initiation of the next

cycle of therapy.

Cycle		1				2- onward						
	Screening	Day 1	Day 8	Day 15	Day 22	Day 1	Day 8	Day 15	Day 22	End of treatment	End of Study (4 weeks after last dose)	Follow-up[j]
Eligibility & Safety Monitoring												
Informed Consent	X[e]											
Demographics	X[e]											
Medical History	X[e]											
Concurrent Medications	X[f]	X[g]	X	X	X	X	X	X	X	X	X	X
Physical exam	X[f]	X[g]	X	X	X	X				X	X	X
Vital Signs, weight	X[f]	X	X	X	X	X				X	X	X
Performance Status	X[f]	X	X	X	X	X			X	X	X	X
CBC w/ differential	X[f]	X[g]	X	X	X	X	X	X	X	X	X	X
Chemistries[a, i]	X[f]	X[g]				X				X	X	X
EKG	X[e]		X				X			X	X	
Toxicity Assessment			X	X	X	X			X	X	X	X
Serum β-hCG	X[f]											
Urinalysis	X[e]	X[g]										
Creatinine clearance	X											
Drug Dispensation												
Oral 5-azacitidine		X				X						
Romidepsin			X	X	X		X	X	X			
Efficacy Measurements												
CT (PET/CT optional)	X[e]						X[b]					
Brain MRI	X[e, l]											
Bone Marrow Biopsy[k]	X									X[c]		
Lymph Node Assessment	X[e]											
PK/Biomarkers												
Lymph Node Biopsy, FNA, or Bone Marrow Biopsy (Optional)	X[e]		X[l]									
PK		X[d]	X[d]			X						
Correlative Blood Sample		X[h]	X[h]	X[h]	X[h]	X[h]						

a) Chemistries include: Chem *, Mg+, K^+, Creatinine, Hepatic Function Panel (LFTs, bilirubin), and LDH. On romidepsin days of cycles 2 and onward, full chemistries may be drawn, but only K^+ and Mg^+ are required.
b) CT to occur after cycle 2 and 6, Then CT q3-6 months, until disease progression or initiation of new therapy at the discretion of the Investigator. (PET/CT is optional)
c) Bone Marrow biopsy will be required after cycle 2 for patients with bone marrow involvement at baseline who have a complete response by imaging and physical exam
d) PK for Oral 5-azacitidine /Romidepsin: See figure 1 for details.
e) To be done within 4 weeks of treatment start date
f) To be done within 1 week of treatment start date
g) Not necessary if conducted within 72 hours of screening assessment
h) PD for Oral 5-azacitidine /Romidepsin: See figure 1 for details.
i) Sodium, potassium, chloride, calcium, and glucose are part of the electrolyte assessment at every time point. Magnesium and phosphorous will be added to the electrolyte panel at Screening, and repeated before administration of romidepsin.
j) Follow-up visits will be every 3 months for 1 year, or until the patient begins a new treatment for their disease for any patient that did not progressed on study.
k) Based on clinical assessment
l) For CNS disease only. Follow up MRI to be done at the discretion of the treating investigator.

ALL SCHEDULED EVENTS +/- 3 DAYS

11. MEASUREMENT OF EFFECT

The primary objective of this study is to evaluate the safety and tolerability of the study drugs. The measurement of toxicity is outlined in Section 7.

Although clinical response is not the primary endpoint of this trial, patients with measurable disease will be assessed by standard criteria. Patients will be re-evaluated at the end of even numbered cycles. Response will be evaluated with physical exam, computerized tomography (CT) and tissue biopsies as defined by the guidelines of the International Harmonization Project Group 2007 Revised Response Criteria. [1] Positron emission tomography / computerized tomography (PET/CT) will be utilized if available (optional). MM patients will be evaluated using criteria described in section 11.2.2

11.1 Evaluation of response:

For the phase I part of the study measurable disease will be utilized as the reference for response. For the phase II part of the study evaluable disease criteria will be utilized for response assessment.

11.1.1 Definitions

Evaluable for toxicity. All patients will be evaluable for toxicity starting from Day 1 of Cycle 1.

Evaluable for objective response. Patients who have received at least one day of therapy, and have had their disease re-evaluated will be considered evaluable for response. These patients will have their response classified according to the definitions stated below. Patients who exhibit objective disease progression prior to the end of cycle 1 will also be considered.

11.1.2 Disease Parameters

Measurable disease. Measurable lesions are defined as those that can be accurately measured in two dimension (longest diameter to be recorded) as $\geq$20 mm with conventional techniques (PET/CT, MRI, x-ray) or as $\geq$10 mm with spiral CT scan. All tumor measurements will be recorded in millimeters (or decimal fractions of centimeters). Tumor volume will be recorded as the sum of the product of the diameters (SPD) of the largest predominant target lesions.

FDG avidity is based on comparison with background tissues. There is no specific SUV value that is considered a cut-off.

Non-measurable disease (evaluable disease). All other lesions (or sites of disease), including small lesions (<10 mm using spiral CT scan), are considered non-measurable disease. Bone lesions, leptomeningeal disease, ascites, pleural/pericardial effusions, lymphangitis cutis/pulmonitis, inflammatory breast disease, abdominal masses (not followed by CT or MRI), and cystic lesions are all non-measurable.

Target lesions. All measurable lesions up to a maximum of 6 lesions total, representative of all involved organs, should be identified as target lesions and recorded and measured at baseline. Target lesions should be selected on the basis of their SUV avidity (High SUV lesions will be prioritized, even if not the largest lesions) and size (lesions with the largest SPD diameter) and their suitability for accurate repeated measurements (either by imaging techniques or clinically). An SPD for all target lesions will

be calculated and reported as the baseline sum SPD. The baseline sum SPD will be used as reference by which to characterize the objective tumor response based on CT criteria.

Non-target lesions. All other lesions (or sites of disease) including any measurable lesions over and above the 6 target lesions should be identified as non-target lesions and should also be recorded at baseline. Measurements of these lesions are not required, but the presence or absence of each should be noted throughout follow-up.

11.1.3 Methods for Evaluation of Measurable Disease in NHL

All measurements should be taken and recorded in metric notation using a ruler or calipers. All baseline evaluations should be performed as closely as possible to the beginning of treatment and never more than 1 week before the beginning of the treatment.

The same method of assessment and the same technique should be used to characterize each identified and reported lesion at baseline and during follow-up. Imaging-based evaluation is preferred to evaluation by clinical examination when both methods have been used to assess the antitumor effect of a treatment.

Clinical lesions Clinical lesions will only be considered measurable when they are superficial (e.g., skin nodules and palpable lymph nodes). In the case of skin lesions, documentation by color photography, including a ruler to estimate the size of the lesion, is recommended.

CT, PET/CT and MRI These techniques should be performed with cuts of 10 mm or less in slice thickness contiguously. Spiral CT should be performed using a 5 mm contiguous reconstruction algorithm. This applies to tumors of the chest, abdomen, and pelvis. Head and neck tumors and those of extremities usually require specific protocols.

Ultrasound (US) Will not be used for disease assessment

11.2 Response Criteria

11.2.1 Evaluation of Measurable Disease in NHL

Table 10 outlines the response criteria used to evaluate disease response to therapy.

Table 10 : Response Criteria for NHL

Response	Definition	Nodal Masses	Spleen/Liver	Bone Marrow CR
CR	Disappearance of all evidence of disease	a) FDG-avid or PET positive prior to therapy; mass of any size permitted if PET negative (b) Variably FDG-avid or PET negative; regression to normal size on CT	Not palpable, nodules disappeared	Infiltrate cleared on repeat biopsy; if indeterminate by morphology, immunohistochemistry should be negative
PR	Regression of measurable disease and no new sites	50% decrease in SPD of up to 6 largest dominant masses; no increase in size of other nodes	50% decrease in SPD of nodules (for single nodule in	Irrelevant if positive prior to therapy; cell type should be specified

		(a) FDG-avid or PET positive prior to therapy; one or more PET positive at previously involved site (b) Variably FDG-avid or PET negative; regression on CT	greatest transverse diameter); no increase in size of liver or spleen	
SD	Failure to attain CR/PR or PD	(a) FDG-avid or PET positive prior to therapy; PET positive at prior sites of disease and no new sites on CT or PET (b) Variably FDG-avid or PET negative; no change in size of previous lesions on CT		
Relapsed or PD	Any new lesion or increase by 50% of previously involved sites from nadir	Appearance of a new lesion(s) 1.5 cm in any axis, 50% increase in SPD of more than one node, or 50% increase in longest diameter of a previously identified node 1 cm in short axis Lesions PET positive if FDG-avid lymphoma or PET positive prior to therapy	50% increase from nadir in the SPD of any previous lesions	New or recurrent involvement
Abbreviations: CR, complete remission; FDG, [18F]fluorodeoxyglucose; PET, positron emission tomography; CT, computed tomography; PR, partial remission; SPD, sum of the product of the diameters; SD, stable disease; PD, progressive disease.				

11.2.3 Response Definitions

Complete Response (CR): Disappearance of all non-target lesions by PET/CT

Incomplete Response/Stable Disease (SD): Persistence of one or more non-target lesion(s) and/or maintenance of tumor marker level above the normal limits

Progressive Disease (PD): Appearance of one or more new lesions and/or unequivocal progression of existing non-target lesions

Although a clear progression of "non-target" lesions only is exceptional, the opinion of the treating physician should prevail in such circumstances, and the progression status should be confirmed at a later time by the review panel (or Principal Investigator).

11.2.4 Evaluation of Best Response

The best overall response is the best response recorded from the start of the treatment until disease progression/recurrence (taking as reference for progressive disease the smallest measurements recorded since the treatment started). The patient's best response assignment will depend on the achievement of both measurement and confirmation criteria.

11.2.5 Overall Response Rate

Overall Response Rate (OR) = CR + PR based on evaluation of best response in each patient.

12. DATA REPORTING / REGULATORY REQUIREMENTS

Adverse event lists, guidelines, and instructions for AE reporting can be found in Section 7.0 (Adverse Events: List and Reporting Requirements).

12.1 Data Reporting

12.1.1 Monitoring

The Institutional Review Board (IRB) at Columbia University Medical Center will monitor this study.

12.1.2 Responsibility for Data Submission

The Study Coordinator is responsible for compiling data for all participants and for providing the data to the Principal Investigator for review.

12.2 Data Safety Monitoring Board

The Herbert Irving Comprehensive Cancer Center at Columbia University Medical Center's Data Safety Monitoring Board (DSMB) will oversee conduct of the study, patient safety and all interim analyses as specified in the data analysis plan. Detailed guidelines regarding the structure, function and decision-making mechanisms for the Data Safety Monitoring Board are provided in the DSMB charter.

12.3 Investigator Reporting Responsibilities

The conduct of the study will comply with all FDA safety reporting requirements.

An IND is not needed for this study as both agents are commercially available.

12.4 Study auditing

Investigator responsibilities are set out in the ICH guideline for Good Clinical Practice (GCP) and in the US Code of Federal Regulations.

Investigators must enter study data onto CRFs or other data collection system. The Investigator will permit study-related audits by Celgene or its representatives, IRB/EC review, and regulatory inspection(s) (e.g., FDA, EMEA, TPP), providing direct access to the facilities where the study took place, to source documents, to CRFs, and to all other study documents.

12.5 Protocol amendments

Any amendment to this protocol must be agreed to by the Principal Investigator and reviewed by Celgene. Amendments should only be submitted to IRB/EC after consideration of Celgene review. Written verification of IRB/EC approval will be obtained before any amendment is implemented.

12.6 Protocol deviations

When an emergency occurs that requires a deviation from the protocol for a subject, a deviation will be made only for that subject. A decision will be made as soon as possible to determine whether or not the subject (for whom the deviation from protocol was effected) is to continue in the study. The subject's medical records will completely describe the deviation from the protocol and state the reasons for such deviation. In addition, the Investigator will notify the IRB/EC in writing of such deviation from protocol.

Non-emergency minor deviations from the protocol will be permitted with approval of the Principal Investigator.

13. STATISTICAL CONSIDERATIONS

13.1 Study Design/ Primary Endpoints

Phase I

This is an open label, single institution Phase I, 3-3 dose-escalation study. The primary objective is to determine the MTD of oral 5-azacitidine in combination with romidepsin. The safety and toxicity of this combination will be evaluated.

All patients who receive any amount of drug will be available for toxicity. Adverse events will be graded using the NCI CTCAE v4.0.

The dose escalation scheme will follow the guideline in Section 5.1.1.

Toxicities will be described by intensity at each dose level

Phase II

Phase 2 will consist of the combination of oral 5-azacitidine + romidepsin in patients with PTCL. (See figure 2 for study plan flow chart)

Exploratory Cohort

The Exploratory Cohort will be descriptive in nature.

13.2 Sample Size/Accrual Rate

Phase I

An estimated 36 patients (max of 45) will be accrued during dose escalation as outlined in Figure 2. Once the MTD is reached 9 further patients will be enrolled at the MTD dose.

The MTD is defined as the level at which $\leq$1 out of 3 or $\leq$2 out of 6 patients have DLTs at highest dose level below the maximally administered dose.

Phase II

Once the initial phase is complete, the phase II will open to allow for a total accrual of 24 patients.

We estimate accrual of an average of 4 patients per month, with a goal of completing accrual within 12 months.

Based on an optimal 2 - stage design, we can test the null hypothesis that the overall response rate is $\leq$25% versus the alternative that the overall response rate is $\geq$50%. If the combination is actually not effective, there is a 0.049 probability of concluding that the combination is effective (target alpha = 0.05); if the combination is actually effective, there is a 0.2 probability of concluding that is ineffective (power = 80%). With 9 patients treated in the first stage, if 2 or fewer patients have a response within a stratum, the trial will be terminated. If the trial continues to the second stage, an additional 15 patients will be studied. If 9 or fewer patients respond by the end of the second stage, the combination will not be considered for further study. The total number of patients studied will be 24. The probability of early

stopping for this trial is 0.60. The expected sample size when the true response rate is 0.25 is 15.

Exploratory Cohort
A total of 10 patients with GC-derived lymphoma will be accrued.

We estimate an average accrual of 1-2 patients per month, with a goal of completing accrual within 12 months.

13.3 Analysis of Secondary Endpoints

Phase I
The maximum number of cycles will be described by dose level.

The number of delays >7 days will be described at the MTD.

ORR as defined by the guidelines of the International Harmonization Project Group 2007 Revised Response Criteria, and will be tabulated by dose level. (See Section 11.2.1.) Response rates will be based on all patients who receive at least one day of medication.

Phase II and Exploratory Cohort
Primary Objective: To estimate ORR defined as best response by 4 cycles.

Disease and patient characteristics at baseline will be summarized using descriptive statistics. For qualitative variables, frequency distributions and proportions will be provided; for quantitative variables, summary statistics (e.g., mean, median, quartiles, standard deviations, etc) and graphical displays (e.g., box plots). Overall response rates will be estimated upon completion of the study along with exact 95% confidence intervals.

REFERENCES

1. Cheson, B.D.,et al., *Revised response criteria for malignant lymphoma.*J Clin Oncol, 2007. **25**(5):p.579-86.
2. Jemal, A., et al., *Cancer statistics, 2004.* CA Cancer J Clin, 2004. **54**(1): p. 8-29.
3. Morton, L.M., et al., *Lymphoma incidence patterns by WHO subtype in the United States, 1992-2001.* Blood, 2006. **107**(1): p. 265-76.
4. Piekarz, R.L., et al., *Phase II multi-institutional trial of the histone deacetylase inhibitor romidepsin as monotherapy for patients with cutaneous T-cell lymphoma.* J Clin Oncol, 2009. **27**(32): p. 5410-7.
5. Malik, S., et al., *FolotynTM (Oral 5-azacitidine Injection) for the Treatment of Patients with Relapsed or Refractory Peripheral T-Cell Lymphoma: Food and Drug Administration Drug Approval Summary.* Clin Cancer Res, 2010.
6. Jagannath,S.,M.A.Dimopoulos,and S.Lonial,*Combined proteasome and histone deacetylase inhibition: A promising synergy for patients with relapsed/refractory multiple myeloma.*Leuk Res,2010.**34**(9): p. 1111-8.
7. O'Connor, O.A., et al., *Phase II-I-II study of two different doses and schedules of oral 5-azacitidine, a high-affinity substrate for the reduced folate carrier, in patients with relapsed or refractory lymphoma reveals marked activity in T-cell malignancies.* J Clin Oncol, 2009. **27**(26): p. 4357-64.
8. DeGraw, J.I., et al., *Synthesis and antitumor activity of 10-propargyl-10-deazaaminopterin.* J Med Chem, 1993. **36**(15): p. 2228-31.
9. Wang, E.S., et al., *Activity of a novel anti-folate (PDX, 10-propargyl 10-deazaaminopterin) against human lymphoma is superior to methotrexate and correlates with tumor RFC-1 gene expression.* Leuk Lymphoma, 2003. **44**(6): p. 1027-35.
10. Kastrup, I.B., et al., *Genetic and epigenetic alterations of the reduced folate carrier in untreated diffuse large B-cell lymphoma.* Eur J Haematol, 2008. **80**(1): p. 61-6.
11. Zain, J. and O. O'Connor, *Oral 5-azacitidine: basic understanding and clinical development.* Expert Opin Pharmacother, 2010. **11**(10): p. 1705-14.
12. Thompson, C.A., *FDA approves oral 5-azacitidine for treatment of rare lymphoma.* Am J Health Syst Pharm, 2009. **66**(21): p. 1890.
13. Marchi, E., et al., *Oral 5-azacitidine Is Synergistic with the Proteasome Inhibitor Bortezomib in In vitro and In vivo Models of T-Cell Lymphoid Malignancies.* Clin Cancer Res, 2010. **16**(14): p. 3648-58.
14. Johnstone, R.W., *Histone-deacetylase inhibitors: novel drugs for the treatment of cancer.* Nat Rev Drug Discov, 2002. **1**(4): p. 287-99.
15. Marks, P., et al., *Histone deacetylases and cancer: causes and therapies.* Nat Rev Cancer, 2001. **1**(3): p. 194-202.
16. Grunstein, M., *Histone acetylation in chromatin structure and transcription.* Nature, 1997. **389**(6649): p. 349-52.
17. Furumai, R., et al., *FK228 (depsipeptide) as a natural prodrug that inhibits class I histone deacetylases.* Cancer Res, 2002. **62**(17): p. 4916-21.
18. Piekarz, R.L. and S.E. Bates, *Epigenetic modifiers: basic understanding and clinical development.* Clin Cancer Res, 2009. **15**(12): p. 3918-26.
19. Bates, S.E., et al., *Laboratory correlates for a phase II trial of romidepsin in cutaneous and peripheral T-cell lymphoma.* Br J Haematol, 2010. **148**(2): p. 256-67.
20. Byrd, J.C., et al., *Depsipeptide (FR901228): a novel therapeutic agent with selective, in vitro activity against human B-cell chronic lymphocytic leukemia cells.* Blood, 1999. **94**(4): p. 1401-8.
21. Imesch, P., D. Fink, and A. Fedier, *Romidepsin reduces histone deacetylase activity, induces acetylation of histones, inhibits proliferation, and activates apoptosis in immortalized epithelial endometriotic cells.* Fertil Steril, 2010.
22. Woo, S., et al., *Population pharmacokinetics of romidepsin in patients with cutaneous T-cell lymphoma and relapsed peripheral T-cell lymphoma.* Clin Cancer Res, 2009. **15**(4): p. 1496-503.
23. Byrd, J.C., et al., *A phase 1 and pharmacodynamic study of depsipeptide (FK228) in chronic lymphocytic leukemia and acute myeloid leukemia.* Blood, 2005. **105**(3): p. 959-67.
24. Klimek, V.M., et al., *Tolerability, pharmacodynamics, and pharmacokinetics studies of depsipeptide (romidepsin) in patients with acute myelogenous leukemia or advanced myelodysplastic syndromes.* Clin Cancer Res, 2008. **14**(3): p. 826-32.
25. Piekarz R, W.J., Frye R, *Results of a phase 2 NCI multicenter study of romidepsin in patients with relapsed peripheral T-cell lymphoma (PTCL) [abstract].* . ASH Annual Meeting Abstracts 2008, 2008. **112**(1567).
26. Hartlapp, I., et al., *Depsipeptide induces cell death in Hodgkin lymphoma-derived cell lines.* Leuk Res, 2009. **33**(7): p. 929-36.
27. *StatBite: FDA oncology drug product approvals in 2009.* J Natl Cancer Inst, 2010. 102(4): p. 219.

Appendix 1: Medications That May Cause QTc Prolongation

The following table presents a list of drugs that may prolong the QTc. These drugs are prohibited during the study. Romidepsin may be administered after a 5 half-life washout period elapses following the use of these drugs. Washout period is based on roughly 5 half-lives and rounded to a convenient interval.

Medications That May Cause QTc Prolongation

Compound (Brand Name)	Compound Half-Life	Possible Washout Period (Hours)	Possible Washout Period (Days)
Antiarrhythmics			
Amiodarone (Cordarone, Pacerone)	58 days (15-142) 36 days (active metabolite)		180
Disopyramide (Norpace, Norpace CR)	6.7 hr (4-10)	36	
Dofetilide (Tikosyn)	10 hr	48	
Flecainide (Tambocor)	20 hr (12-27)		5
Ibutilide (Corvert)	6 hr (2-12) (variable among patients)	36	
Procainamide (Pronestyl, Procanbid, Procan)	3-4 hr for PA and NAPA (active metabolite)	24	
Quinidine (Quinaglute, Cardioquin, Quinidex)	6-8 hr in adult; 3-4 hr in children	36	
Sotalol (Betapace, Sorine)	12 hr	72	
Antibiotics			
Clarithromycin (Biaxin, Biaxin XL)	Nonlinear PK 3-4 hr (250 mg Q12) 5-7 hr (500 mg Q12)	36	
Erythromycin (Benzamycin, Eyrc, E-glades, Erygel, E-solve 2, Akne-Mycin, Eryderm, Sansac, Erythro-Statin, Erymax, Staticin, T-Stat, C-solve-2, Erycetter, PCE, Ery-Tab, E-Mycin, E-Base, E.E.S., Eryped, E.E.S 200, E.E.S 400, Pediamycin, Eryzole, Erythrocin)	Each salt form has different half-life		
Gatifloxacin (Tequin, Tequin Teqpaq)	7-14 hr	48	
Grepafloxacin (Raxar)	16 hr		3
Antibiotics *(cont'd)*			
Levofloxacin (Levaquin, Quixin, Elequin)	6-8 hr	48	
Moxifloxacin (Avelox, Vigamox)	12 ± 1.3 hr	72	
Sparfloxacin (Zagam)	20 hr (16-30)		4
Telithromycin (Ketex)	2-3 hr	24	
Anticonvulsants			
Felbamate (Felbatol)	20-23 hr		5
Fosphenytoin (Cerebyx)	12-29 hr		6
Antidepressants			
Venlaflaxine (Effexor)	5 ± 2 hr for parent comp. 11± 2 hr for OVD (active metabolite)	60	
Antidiarrheals			
Octreotide (Sandostatin)	1.7 hr	12	
Antiemetics			
Dolasetron (Anzemet)	8.1 hr		
Droperidol (Inapsin)	2.2 hr	10	
Domperidone (Motilium)	7-8 hr	48	
Palonosetron (Aloxi)	40 hr		10
Antihypertensives			
Moexipril/Hydrochlorothiazide (Uniretic)	2-9 hr(include active metabolite) for moexipril; 5.6-14.8 hr for HCTZ	48	
Antimalarials			
Halofantrine (Halfan)	6-10 days (variable among individuals)		45
Quinidine (Quinaglute, Cardioquin, Quinidex)	6-8 hr in adult; 3-4 hr in children	36	
Antimanics			
Lithium (Eskalith, Lithobid, Lithonate)	24 hr (10-50)		7
Antineoplastics			

Compound (Brand Name)	Compound Half-Life	Possible Washout Period (Hours)	Possible Washout Period (Days)
Arsenic trioxide (Trisenox)	Not characterized		
Tamoxifen (Nolvadex)	5-7 days (biphasic)		30
Antiprotozoals			
Pentamidine (NebuPent, Pentam)	6.4 ± 1.3 hr	36	
Antipsychotic agents			
Chlorpromazine (Thorazine)	30 ± 7 hr		7
Haloperidol (Haldol)	18 ± 5 hr		5
Mesoridazine (Serentil)	24-48 hr (animal study)		10
Pimozide (Orap)	55 hr		14

(Continued from previous page)

Antipsychotic agents *(cont'd)*			
Quetiapine (Seroquel)	6 hr	36	
Risperidone (Risperdal, Risperdal Consta)	3-20 hr (extensive to poor metabolizer) 9-hydroxyrisperidone (active metabolite) T ½ = 21-30 hr (extensive to poor metabolizer)		4
Thioridazine (Mellaril)	20-40 hr (Phenothiazines)		7
Ziprasidone (Geodon, Zeldox)	7 hr	36	
Antispastics			
Tizanidine (Zanaflex)	2.5 hr	12	
Antivirals			
Amantadine (Symadine, Symmetrel)	17 ± hr (10-25)		4
Foscarnet (Foscavir)	87.5 ± 41.8 hr (distribution and release from bone)		20
Analgesics			
Levomethadyl (Orlaam)	Multiple compartment PK with active metabolite 2.6 day for LAAM, 2 day for nor-LAAM, 4 day for dinor-LAAM		20
Asthma medications			
Salmeterol (Advair Diskus, Serevent, Serevent Diskus)	5.5 hr (only one datum)	36	
Calcium channel blockers			
Bepridil (Vascor)	42 hr (26-64)		10
Isradipine (DynaCirc)	8 hr (multiple metabolites)	48	
Nicardipine (Cardene)	~2 hr post IV infusion	12	
Cholinergic enhancers			
Cisapride (Propulsid)	6-12 hr, up to 20 hr	60	
Diuretics			
Indapamide (Lozol)	14 hr (biphasic elimination)		3
Immunosuppressants			
Tacrolimus (Prograf, Protopic)	~34 hr in healthy patients ; ~19 hr in kidney transplant		7
Migraine medications			
Naratriptan (Amerge)	6 hr	36	
Sumatriptan (Imitrex)	2.5 hr	12	
Zolmitriptan (Zomig)	2.8-3.7 hr (higher in female)	18	
Narcotic pain relievers			

Compound (Brand Name)	Compound Half-Life	Possible Washout Period (Hours)	Possible Washout Period (Days)
Methadone (Dolophine, Methadose)	15-30 hr		7
Sedatives			
Chloral hydrate	Readily converted to Trichloroethanol (active metabolite T½= 7-10 hour)	48	

References:
Physician's Desk Reference 2002
Facts and Comparisons (update to June, 2000)
The Pharmacological Basis of Therapeutics 9th Edition, 1996

Appendix 2: Medications That May Inhibit CYP3A4

The following table presents a list of drugs that may inhibit CYP3A4. As romidepsin is predominately metabolized by CYP3A4, inhibition of this enzyme could result in elevated plasma levels or increased exposure to romidepsin. Romidepsin may be administered after a 5 half-life washout period elapses following the use of these drugs. Washout period is based on roughly 5 half-lives and rounded to a convenient interval.

Medications That May Inhibit CYP3A4

Compound (Brand Names)	Compound Half-Life	Possible Washout Period - Hours	Possible Washout Period - Days
Azole Antifungals			
Clotrimazole (Mycelex, Lotrimin, Lotrisone)	Not available		
Ketoconazole (Nizoral, Ketozole)	6 hr (2-8 hr)	30 hr	
Itraconazole (Sporanox)	21 hr		5 days
Fluconazole (Diflucan)	3 hr	15 hr	
Miconazole (Monistat)	57 hr		11 days
HIV Protease Inhibitors			
Ritonavir (Norvir, Kaletra)	4 hr	20 hr	
Indinavir (Crixivan)	2 hr	10 hr	
Saquinavir (Invirase, Fortovase)	5 hr	25 hr	
Nelfinavir (Viracept)	4 hr	20 hr	
Delavirdine (Rescriptor)	6 hr	30 hr	
Macrolide Antibiotics			
Troleandomycin (Tao)	Not available		
Erythromycin (Benzamycin, Eyrc, E-glades, Erygel, E-solve 2, Akne-Mycin, Eryderm, Sansac, Erythro-Statin, Erymax, Staticin, T-Stat, C-solve-2, Erycetter, PCE, Ery-Tab, E-Mycin, E-Base, E.E.S., Eryped, E.E.S 200, E.E.S 400, Pediamycin, Eryzole, Erythrocin)	2 hr	10 hr	
Clarithromycin (Prevpac, Biaxin)	5 hr	25 hr	
Other Antibiotics			
Chloramphenicol (Chloromycetin, Chloroptic)	4 hr	20 hr	
Ciprofloxacin (Ciprodex, Cipro, Ciloxan)	4 hr	20 hr	
Norfloxacin (Noroxin, Chibroxin)	4 hr	20 hr	
Serotonin Reuptake Inhibitors (SSRI's)			
Fluoxetine (Prozac, Sarafem, Symbyax)	Fluoxetine 5 days Norfluoxetine (active metabolite) 12 days (4-16 days)		60 days
Nefazodone (Serzone)	3 hr	15 hr	
Fluvoxamine (Luvox)	16 hr		3 days
Antiemetics			
Aprepitant (Emend)	11 hr (9-13 hr)		2 days
Oral Contraceptives			
Ethinyl-estradiol (Kariva, Velivet, Mircette, Desogen, Cyclessa, Ortho-Cept, Yasmin, Demulen, Zovia, NuvaRing, Seasonale, Lessina, Portia, Levlite, Nordette, Aviane, Enpresse, Trivora, Levora, Alesse, Triphasil, Ortho Evra, Ovcon, Nortrel, Gencept, Balziva, Brevicon, Norinyl, Norethin, Aranelle, Ortho-Novum, Modicon, Tri-Norinyl, Femhrt, Junel, Loestrin, Estrostep, Microgestin, Tri-Previfem, Previfem, Tri-Sprintec, Sprintec, Ortho Tri-Cyclen, Cryselle, Low-ogestrel, Ogestrel, Lo/Ovral,	15 hr		3 days

Compound (Brand Names)	Compound Half-Life	Possible Washout Period - Hours	Possible Washout Period - Days
Ovral)			
Oral Contraceptives *(cont'd)*			
Mifepristone (Mifeprex, RU-486)	18 hr		4 days
Gestodene	20-22 hr		5 days
Histamine H2-Receptor Antagonists			
Cimetidine (Tagamet)	2 hr	10 hr	
Antiarrhythmic Drugs			
Quinidine (Quinaglute, Cardioquin, Quinidex)	7 hr	35 hr	
Amiodarone (Cordarone, Pacerone)	53 days (15-142 days)		265 days
Antihypertensives			
Diltiazem (Taztia, Cartia, Cardizem, Dilt-CD, Dilacor, Teczem, Tiamate. Trizac)	3 hr [7 hr for extended release (Trizac)]	15 hr 35 hrs	
Verapamil (Tarka, Verelan, Isoptin, Covera-HS, Calan)	8 hr	40 hr	
Calcium Channel Blocker			
Mibefradil (Posicor)	21 hr (17-25 hr)		5 days
Others			
Grapefruit juice	Not available		
Star fruit	Not available		

Appendix 3 Canadian Cardiovascular Society Angina Classification

Class I
Ordinary physical activity, (e.g., walking and climbing stairs) does not cause angina; angina occurs with strenuous, rapid, or prolonged exertion at work or recreation.
Class II
Slight limitation of ordinary activity; angina occurs on walking or climbing stairs rapidly; walking uphill; walking or stair climbing after meals, in cold, in wind, or under emotional stress; or only during the few hours after awakening; when walking > 2 blocks on level ground; or when climbing more than 1 flight of stairs at a normal pace and in normal conditions.
Class III
Marked limitation of ordinary physical activity; angina occurs on walking 1 to 2 blocks on level ground or climbing 1 flight of stairs at a normal pace in normal conditions.
Class IV
Inability to perform any physical activity without discomfort; anginal symptoms may be present at rest.

Campeau L. Grading of angina pectoris. Circulation 1975;54:522-3.

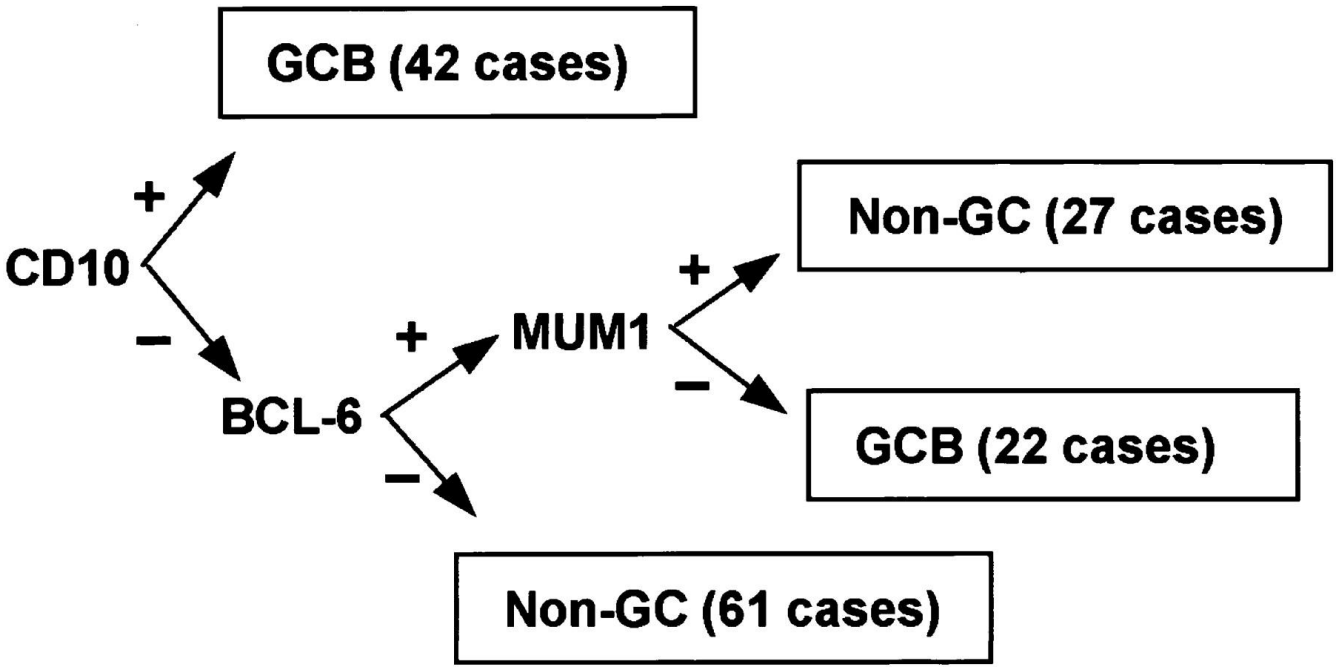
Appendix 4 New York Heart Association Classification of Cardiac Disease

NYHA Classification of Cardiac Disease

Class	Functional Capacity	Objective Assessment
I	Patients with cardiac disease but without resulting limitations of physical activity. Ordinary physical activity does not cause undue fatigue, palpitation, dyspnea, or anginal pain.	No objective evidence of cardiovascular disease.
II	Patients with cardiac disease resulting in slight limitation of physical activity. They are comfortable at rest. Ordinary physical activity results in fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of minimal cardiovascular disease.
III	Patients with cardiac disease resulting in marked limitation of physical activity. They are comfortable at rest. Less than ordinary activity causes fatigue, palpitation, dyspnea, or anginal pain.	Objective evidence of moderately severe cardiovascular disease.
IV	Patients with cardiac disease resulting in inability to carry on any physical activity without discomfort. Symptoms of heart failure or the anginal syndrome may be present even at rest. If any physical activity is undertaken, discomfort is increased.	Objective evidence of severe cardiovascular disease.

Source: The Criteria Committee of New York Heart Association. Nomenclature and Criteria for Diagnosis of Diseases of the Heart and Great Vessels. 9th Ed. Boston, MA: Little, Brown & Co; 1994:253-256.

Appendix 5: Hans Criteria



Christine P. Hans et al. Blood 2004;103:275-282