Amendment

Protocol Number: 07C0081-L **Reference Number:** 373915

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Protocol Title: A Phase I/II Study of Flavopiridol in Relapsed or Refractory Mantle Cell Lymphoma (MCL) and Diffuse Large B-cell Lymphoma (DLBCL)

SIGNATURES

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PI is the Accountable Investigator

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N/A

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N/A

For Institute/Center Scientific Review Committee:

N/A

Other IC Clinical Director signatures:

N/A

APPROVALS

IRB Chair:

Michael Hamilton - applied signature on 10/07/2017 2:50 AM EDT

Clinical Director:

N/A

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OPS Protocol Specialist:

Lemuel Clayborn	AM L	10/16/2017
Signature	Print Name	Date

* Signature signifies that investigators on this protocol have been informed that the collection and use of personally identifiable information at the NIH are maintained in a system of record governed under provisions of the Privacy Act of 1974. The information provided is mandatory for employees of the NIH to perform their assigned duties as related to the administration and reporting of intramural research protocols and used solely for those purposes. Questions may be addressed to the Protrak System Owner.

** I have reviewed this research project and considered the NIH Policy for Inclusion of Women and Minorities in Clinical Research. Taking into account the overall impact that the project could have on the research field involved, I feel the current plans adequately includes both sex/gender, minorities, children, and special populations, as appropriate. The current enrollment is in line with the planned enrollment report for inclusion of individuals on the basis of their sex/gender, race, and ethnicity and is appropriate and of scientific and technical merit.

IRB Meeting Date: Expedited

DEC Clearance Date: NA

Protocol Version Date: 09/28/2017

Abbreviated Title: Flavo in relapsed lymphomas
CC Protocol #: 07C0081 L
CTEP Protocol #: #7844 (NOTE: Completed with CTEP; Amendment J, dated 01/16/2015)
Version Date: 09/28/2017
NCT Number: NCT00445341

A Phase I/II Study of Flavopiridol in Relapsed or Refractory Mantle Cell lymphoma (MCL) and Diffuse Large B-cell Lymphoma (DLBCL)

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Investigator Roles:

A. Obtain information by intervening or interacting with living individuals for research purposes
B. Obtaining identifiable private information about living individuals
C. Obtaining the voluntary informed consent of individuals to be subjects
D. Makes decisions about subject eligibility
E. Studying, interpreting, or analyzing identifiable private information or data/specimens for research purposes
F. Studying, interpreting, or analyzing de-identified data or specimens for research purposes
G. Some/all research activities performed outside NIH

Investigational Agents:

Drug Name:	Flavopiridol
IND Number:	IND #46211
Sponsor:	CTEP
Manufacturer:	Sanofi-Aventis Pharmaceuticals, Inc.

NOTE: The protocol has been completed with CTEP effective Amendment J (version date 01/16/2015).
Commercial Agents: None

PRÉCIS

Background:

- Flavopiridol is a synthetic N-methylpiperidinyl, chlorophenyl flavone compound that targets a number of different cellular pathways and processes.
- It works through several different mechanisms that include inhibition of cyclin dependent kinases and the cyclin D-1 complex which is over-expressed in mantle cell lymphoma. Flavopiridol also has demonstrated activity in activated B-like diffuse large B-cell lymphoma cell lines.
- One of the great challenges in developing flavopiridol and applying it clinically has been determining its optimal dosing schedule. Following several different dosing schedules, one strategy that has been very promising in CLL is the application of so-called hybrid schedules of the drug (an infusion for an intermediate time following a bolus dose).

Objectives:

- Assess the toxicity and safety of administration of this hybrid schedule
- Assess the response rate of the hybrid schedule of flavopiridol in relapsed MCL and DLBCL

Eligibility:

- Relapsed MCL or DLBCL
- ECOG P.S. ≤ 2
- Age ≥ 18 years
- HIV serology negative

Design:

- Phase I/II
- Phase I portion consists of 3-4 dose levels of 3-6 patients each
- Administer weekly X 4 and then 2 weeks off (1 cycle). Restage after every 2 cycles. Continue if CR, PR or SD for up to 6 cycles. Dose reductions for toxicity will be addressed in the protocol.
- Phase II portion of the study will be a Simon optimal two-stage design: designed to rule out 20% response rate (p0=0.20) in favor of a 45% response rate (p1=0.45)
- The maximum sample size to be accrued for this study will be 71 patients.

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1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary

- Assess the response rate of flavopiridol in patients with relapsed mantle cell lymphoma (MCL) and diffuse large B-cell lymphoma (DLBCL) when administered as a 30 minute loading dose followed by a 4-hour infusion once weekly for 4 consecutive weeks every 6 weeks
- Assess the toxicity profile, dose-limiting toxicity and maximum tolerated dose of flavopiridol administered in the hybrid schedule of a 30 minute loading dose followed by a 4-hour infusion once weekly for 4 consecutive weeks every 6 weeks in patients with relapsed MCL and DLBCL.

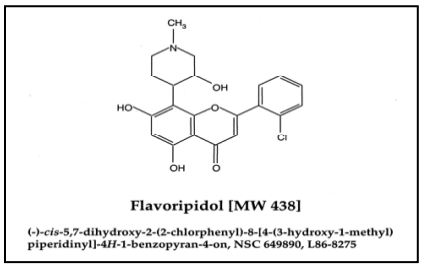
1.1.2 Secondary

- Determine the pharmacokinetics of flavopiridol when administered by hybrid schedules, correlating this with toxicity and Tumor Lysis Syndrome (TLS).
- Assess tissue from tumor biopsies (where possible before and after flavopiridol) by microarray and protein analysis.

1.2 BACKGROUND AND RATIONALE

1.2.1 Flavopiridol Background

Flavopiridol is a synthetic flavone compound with a novel structure, compared to polyhydroxylated flavones such as quercetin and genistein.



Flavoripidol [MW 438]

(-)-*cis*-5,7-dihydroxy-2-(2-chlorphenyl)-8-[4-(3-hydroxy-1-methyl) piperidinyl]-4*H*-1-benzopyran-4-on, NSC 649890, L86-8275

Flavopiridol is obtained from a synthetic process, but its chemical structure is identical to a product obtained from an indigenous plant in India called *Dysoxylum binectariferum*. When flavopiridol was first discovered, it was considered to be a tyrosine kinase antagonist but subsequently, it was shown to reversibly inhibit growth via inhibition of cyclin-dependent kinase (CDK)1 and CDK2[1, 2]. Flavopiridol induced cell cycle inhibition by altering phosphorylation of tyrosine residues as well as antagonizing CDK1 and CDK2 activity as a result of competitive inhibition with ATP[3]. Based on these initial observations, investigators hypothesized that flavopiridol would be effective in rapidly dividing tumor systems where a minimum volume of tumor exists. We now know that flavopiridol has a number of additional targets that include the cyclin D-1 complex over-expressed in mantle cell lymphoma and cyclin H. In addition, studies in lymphoma cell lines have demonstrated activity against activated B-cell (ABC) type cell lines and NF-kappa B down-regulation[4, 5].

1.2.2 Dose Scheduling of Flavopiridol

One of the great challenges in developing flavopiridol and applying it in clinical trials has been its dose scheduling. Initial in vitro work performed at the NCI evaluated a long continuous infusion administration (72 hours: 50 mg/m^2/day every 2 weeks)[6]. 72 hours exposure resulted in growth inhibition with little cell recovery over the next 3 days. These experiments provided support for a 72 hour infusion schedule that was subsequently utilized in later phase I studies. Although patients achieved steady state concentrations that had been shown to be cytotoxic in vitro, the response rate in solid tumor and lymphoma patients in the initial Phase I studies was disappointing. This is likely explained by the fact that the effects of flavopiridol are far more potent in fetal calf serum (used for most in vitro experiments) than in human plasma because of high protein binding in human (95-98%) compared to bovine (0%) serum. thus, much higher concentrations appear to be needed in vivo to achieve adequate free drug levels.

Following this realization, alternative schedules of administration were investigated. In leukemia and lymphoma models, bolus schedules that achieved high concentrations resulted in potent anti-tumor effects. A marked *in vivo* dose response curve with bolus administration of flavopiridol, compared to 72 hour continuous exposure was observed by Sausville and colleagues[7]. Trials in mantle cell lymphoma and CLL demonstrated that daily bolus schedules induced partial responses compared to long continuous infusions[8-10]. However, the half-life of flavopiridol is short following 1-hour administrations (3-4 hours) and brief exposure to this high concentration is probably inadequate to inhibit cellular targets. One strategy that could potentially produce high concentrations of drug for a relatively prolonged period of time is an infusion of flavopiridol for an intermediate time following a bolus loading dose.

There has been much interest in investigating these so-called 'hybrid' schedules and at present, Byrd and colleagues are conducting a phase I/II study of the hybrid schedule 30 minute bolus (30 mg/m^2) followed by a 4 hour infusion (30 mg/m^2 on cycle 1 with dose increase to 50 mg/m^2 on subsequent cycles) in patients with previously treated CLL/SLL. To recap, both 24-hour and 72-hour continuous infusion schedules in CLL were associated with 0 response rate and a bolus schedule produced a partial response rate of merely 11%[8]. Thus far, the response rate to the hybrid flavopiridol schedule has been 52% with some dramatic responses in lymph node reduction (Byrd: personal communications). This "step" schedule was adopted to reduce the incidence of severe TLS on cycle 1, which appears to be dose related. The development of TLS has only been seen in patients with CLL who have very high white cell counts (> 100,000). Therefore, given the very different biologies of the tumor types that we will be treating - as the patient population will consist of relapsed mantle cell and diffuse large B-cell lymphoma - we do not expect that TLS will be a significant problem or complication.

1.2.3 Results of Phase I studies of Flavopiridol

The dose limiting toxicity for flavopiridol as a 72-hour continuous infusion schedule repeated every two weeks was secretory diarrhea[6]. Other toxicities included a proinflammatory syndrome with alterations in acute-phase reactants. Additional toxic effects included malaise, fever, hypoalbuminemia, tumor pain, hypotension, pericardial/pleural effusions, fatigue, anorexia, dysgeusia, lightheadedness, minimal nausea and vomiting, and elevated transaminases. The MTD was 50mg/m^2/day for 3 days.

A phase I trial of flavopiridol administered as a 1-hour bolus daily for 3 consecutive days has also been reported[11]. This regimen was repeated every 3 weeks. The dose limiting toxicity occurred at 62.5 mg/m^2 and included short duration neutropenia, diarrhea, nausea and vomiting.

Several phase I combination studies of flavopiridol with other chemotherapy agents have been initiated. Limited data are available from a flavopiridol + paclitaxel combination study utilizing paclitaxel (175 mg/m^2 over 3 hours) followed on day 2 by flavopiridol (as 24 hour infusion) based upon *in vitro* synergy data from solid tumor cell lines[12]. This study included an escalation of flavopiridol to 80 mg/m^2 without demonstrable fatigue or diarrhea[13]. At higher concentrations, reversible pulmonary toxicity was observed that was dose limiting. It is uncertain if dose escalation above 80 mg/m^2 will be possible with the exclusion of paclitaxel.

The hybrid schedule that is being tested by Byrd and colleagues (personal communication) has been very effective in patients with relapsed CLL and SLL. Responses have been rapid with the development of acute tumor lysis syndrome (TLS) in some patients at higher doses with very high white cell counts. Thus far, 23 patients have been treated. The PR rate is 43% with a median response duration of 12 months. 2 patients developed tumor lysis syndrome (these patients had a high white cell count or bulky disease). Since the occurrence of these cases of TLS, patients with very high white cell counts are ineligible for treatment. All patients receive TLS prophylaxis and careful in-patient monitoring. Other grade 3 or 4 toxicities that have been encountered thus far include neutropenia, anemia, thrombocytopenia, hypokalemia, syncope, diarrhea, arthralgia, anemia and fatigue.

1.2.4 Pharmacokinetics of Flavopiridol

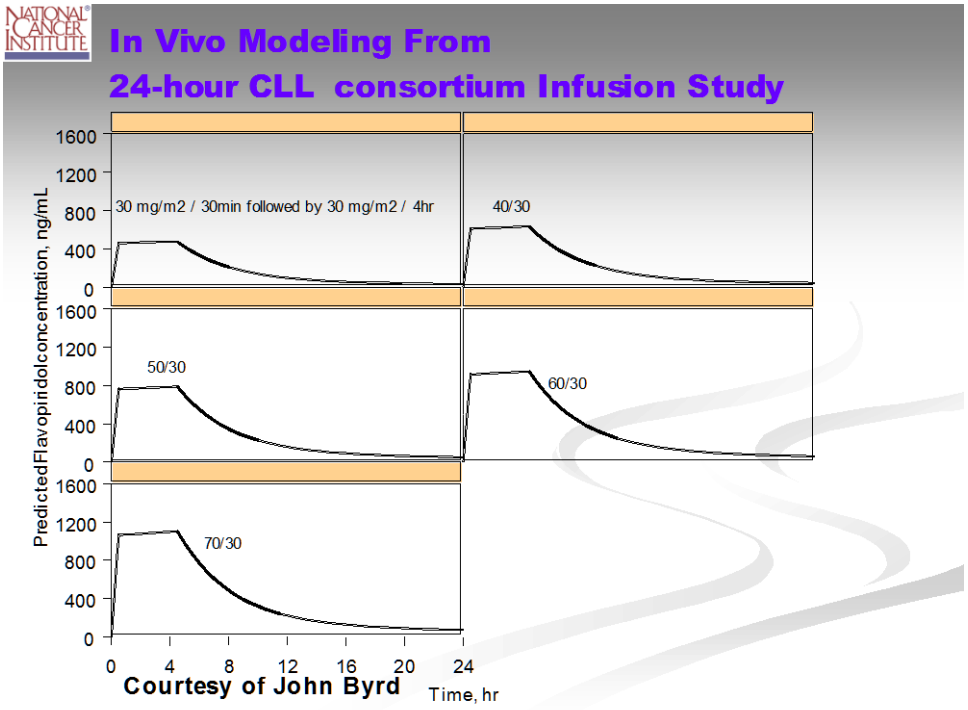
As discussed above, the high plasma protein binding of flavopiridol explains its very low free concentration in human plasma compared to media containing fetal calf serum and explains the lack of clinical responses with various schedules. Studies that have assessed the pharmacokinetics of flavopiridol have shown a very wide variability in clearance and volume of distribution due to plasma protein binding.

The elimination half-life of flavopiridol varies based on the infusion duration:

a) 72 hour infusion: 10-27 hours[11] [14, 15]
b) 24 hour infusion : 22 hours[16]
c) 1 hour infusion: 3.5 to 5 hours[11]

The total body clearance ranges from 11-16 L/hr/m

Simulations of the plasma-concentration time curves at the proposed dose-schedules (courtesy of John Byrd):



We plan to assess and investigate the pharmacokinetics of flavopiridol in this study. We will collect samples at 0.5 , 4.5 and 6 hours following the start of the bolus dose of flavopiridol on doses 1 and 2 of cycle 1. These time-points are based on results from Byrd and colleagues who investigated this hybrid schedule of flavopiridol in patients with CLL.[17]

1.2.5 Rationale for Flavopiridol in MCL and DLBCL

1.2.5.1 MCL

Mantle cell lymphoma (MCL) is an aggressive non-Hodgkin lymphoma (NHL) and constitutes approximately 5-8 % of all NHLs. It usually presents with advanced stage disease, has a median age at diagnosis of approximately 65 years and is considered incurable with chemotherapy and antibody treatment. MCL is characterized by a distinct biologic profile and pathogenesis. The t(11;14) chromosomal translocation and over-expression of the cell cycle regulatory protein cyclin D1 are characteristic molecular findings. Recent advances have identified distinct intracellular pathways that likely play an important role in the pathobiology of MCL. This elucidation has led to the testing of agents that specifically target abnormal pathways in MCL cells. One of the most attractive therapeutic targets for MCL is the cyclin D1 complex which is associated with cell cycle deregulation. Flavopiridol targets a number of different cellular pathways and processes. It induces cell cycle arrest by cyclin dependent kinase (cdk) inhibition and its actions include the inhibition of cdk7 and cyclin H, and the down-regulation of cyclin D1 and D3 expression leading to G1 arrest. Exposure of mantle cell lymphoma cell lines to flavopiridol-induced apoptosis has been associated with decreased levels of cyclin D1 expression. Therefore, investigating flavopiridol in patients with MCL is a very interesting strategy. In MCL, flavopiridol at a dose of 50 mg/m^2/day has been tested both as a 72 hour continuous infusion every 2 weeks and as a 1 hour infusion daily X 3, every 3 weeks[9, 10]. No patients (0/10) responded to the continuous infusion schedule but 3 of 27 patients had a partial response to the bolus schedule (12% PR) – there is no statistical

difference between these two portions (Fisher's exact test, p=0.55). These results suggest that the continuous infusion schedule achieved inadequate steady state levels of drug to induce cytotoxicity and that the bolus schedule probably induced adequate drug levels but for too short a time to achieve significant tumor kill. Thus, our hope is that the hybrid schedule of flavopiridol will achieve high enough and adequate cytotoxic drug levels (bolus) that are maintained for enough time (infusion) to effectively kill tumor cells.

1.2.5.2 DLBCL

DLBCL is also an aggressive disease and the most common subtype of NHL. Gene expression profiling has revealed that there are at least 3 distinct sub-types of DLBCL and one of these, the activated B-cell like (ABC) type, is associated with a worse prognosis and patients who relapse after initial therapy are more likely to have this type. It is particularly worthwhile investigating and developing therapies that can target the ABC type. Studies of flavopiridol lymphoma cell lines have demonstrated strong activity of flavopiridol on OCI-Ly3 lymphoma cell lines which are an excellent model of the ABC type of DLBCL[4]. Flavopiridol inhibits the NF-kappa B pathway which is constitutively activated in the ABC type of DLBCL. Thus, the ABC type of DLBCL may be a particularly attractive target for flavopiridol and therefore our interest in investigating the drug in DLBCL[5].

1.2.5.3 Hybrid schedule in MCL and DLBCL

We propose to evaluate the safety of the hybrid schedule of flavopiridol which is administered as a 30 minute bolus followed by a 4 hour infusion weekly x 4 consecutive weeks, followed by 2 weeks without flavopiridol treatment for a cycle duration of 6 weeks in patients with relapsed or refractory MCL and DLBCL. To determine the safe tolerated dose of the hybrid schedule, a phase I dose escalation will be performed to determine the MTD. To determine the efficacy of the hybrid schedule at the MTD, a phase II study will be performed in MCL and DLBCL.

There are distinct molecular targets amenable to flavopiridol in both MCL and DLBCL, which we plan to evaluate. Among these are the cyclin D-1 complex, NF-kappa B, various cyclin dependent kinases and various specific genes. The rationale for studying these markers is as follows: In mantle cell lymphoma cells, we wish to investigate if markers such as cyclin D-1 and certain cyclin dependent kinases are down-regulated by flavopiridol and if there are any patterns of association between levels of certain markers and outcome. In DLBCL, we will assess in an exploratory manner if flavopiridol is more effective in the ABC versus GCB subtype of DLBCL. Because these studies are exploratory and hypothesis generating, the study will not be powered to answer a specific biological question.

Given the activity of flavopiridol when administered as a bolus schedule, we are interested in investigating the hybrid schedule of Byrd and colleagues that has yielded very promising results in CLL/SLL. We aim to both assess the efficacy of the hybrid schedule in MCL and DLBC and in addition determine if tumor lysis syndrome, which has been reported in approximately 15% of CLL/SLL patients treated with this schedule, occurs in MCL and DLBCL patients.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Previously treated mantle cell lymphoma or diffuse large B-cell lymphoma (to include mediastinal (thymic) large B-cell lymphoma; transformed large B-cell lymphoma; follicular grade IIIB large B-cell lymphoma; intravascular large B-cell lymphoma)

2.1.2 Confirmed pathological diagnosis at the National Cancer Institute, NIH.

2.1.3 Recurrent measurable disease (measurable disease in 2 dimensions or leukemic disease which can be quantified and followed)

2.1.4 Prior anthracycline-based treatment for patients with DLBCL

2.1.5 Age > 18years

2.1.6 ECOG performance 2 or better

2.1.7 Major organ function: ANC > 1000/mcL, Platelet > 50,000/mcL, Creatinine < 1.5 mg/dL or creatinine clearance > 60 mL/min; SGPT < 5 x upper limit of normal; bilirubin < 2 mg/dL (total) except < 5 mg/dL in patients with Gilbert's syndrome as defined by > 80% unconjugated. ANC and platelet requirements must be met independent of transfusion.

2.1.8 Informed consent and willingness to use contraception by both men and women.

2.1.9 Both male and female patients must be willing to use adequate contraception (to include effective barrier methods of contraception) or to completely abstain from heterosexual intercourse while on protocol treatment.

2.1.10 Not pregnant or nursing because of an unknown potential for teratogenic or abortifacient effects

2.1.11 HIV serology negative. HIV positive patients receiving combination anti-retroviral therapy are excluded from the study because of possible pharmacokinetic interactions with flavopiridol. Additionally, the biology of HIV associated DLBCL's is often quite different from HIV negative disease due to involvement of EBV.

2.1.12 Hepatitis B surface antigen negative.

2.1.13 No active CNS lymphoma. These patients have a poor prognosis and because they frequently develop progressive neurological dysfunction that would confound the evaluation of neurological and other adverse events.

2.1.14 No history of inflammatory bowel disease unless this has been inactive for a period of 2 or more years.

2.1.15 Recovery from toxicity of prior therapy to a grade 1 or less.

2.1.16 No systemic cytotoxic or experimental treatments within 4 weeks of treatment.

2.1.17 No WBC > 100,000 cells/mcL

2.2 RESEARCH ELIGIBILITY EVALUATION

Blood tests (except serologies) and pregnancy test to be done within one week of study entry, other studies to be done within 4 weeks before study entry:

- Complete History and Physical examination.
- CBC, differential, PT, PTT, SGOT, SGPT, LDH, alkaline phosphatase, bilirubin, albumin, calcium, phosphate, uric acid, creatinine (24 hour creatinine clearance if serum creatinine > 1.5 mg/dL), electrolytes, urinalysis, HIV serology, hepatitis B surface antigen and Anti Hepatitis B core antibody.
- Unilateral bone marrow aspirate and biopsy if not performed within 1 month.
- HCG (urine) in women of childbearing potential.
- Electrocardiogram.
- Staging: CT scans of chest, abdomen and pelvis.
- PET as clinically indicated.
- Head CT or Brain MRI and lumbar puncture with flow and cytology as clinically indicated.

2.3 PATIENT REGISTRATION

Authorized staff must register an eligible candidate with NCI Central Registration Office (CRO) within 24 hours of signing consent. A registration Eligibility Checklist from the web site (http://camp.nci.nih.gov/ccr/welcome.htm) must be completed and faxed to 301-480-0757. After confirmation of eligibility at Central Registration Office, CRO staff will call pharmacy to advise them of the acceptance of the patient on the protocol prior to the release of any investigational agents. Verification of Registration will be forwarded electronically via e-mail to the research team. A recorder is available during non-working hours.

3 STUDY IMPLEMENTATION

3.1 STUDY DESIGN:

3.1.1 Phase I Design

There will be 3-4 defined dose levels, with 3-6 patients treated at each dose level. The Phase I dose escalation will follow one of two different escalation rates shown in Tables 1 and 2. If DLT is observed in 0-1/6 patients on dose level 1, escalation will proceed according to Table 1. If DLT is observed in 2 of up to 6 patients on dose level 1, escalation will proceed according to Table 2. Escalation decision rules are shown below:

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
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.

1 out of 3	Enter at least 3 more patients at this dose level. • If 0 of these 3 patients experience DLT, proceed to the next dose level. • If 1 or more of this group suffer DLT, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three (3) additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose. This rule does not apply to dose level one. Please see section 4.2
≤1 out of 6 at highest dose level below the maximally administered dose	This is the recommended phase 2 dose. At least 6 patients must be entered at the recommended phase 2 dose.

Entry onto a new dose level cannot occur until all patients on the previous dose level have completed cycle 1. The PI or his designee must be consulted regarding the proper dose level for all new patients. The definition of DLT is described in section 4.2.

3.1.2 Phase 2 Design

The Phase 2 dose and schedule will be determined by the MTD in the Phase 1 portion. Please contact the PI or Study Chair for appropriate dose.

3.2 DRUG ADMINISTRATION

3.2.1 Phase 1 Dosing and Schedule

The Phase I dose escalation will follow one of two different escalation rates shown in Tables 1 and 2 below. If DLT is observed in 0/3 or 1/6 patients on dose level 1, subsequent patients will be enrolled according to the paradigm described in Table 1 below. If DLT is observed in 2/6 patients on dose level 1, subsequent patients will be enrolled according to the paradigm described in Table 2 below.

Flavopiridol dose levels

Table 1	Cycle 1, Week 1		Cycle 1, Weeks 2–4, AND All weeks During All Subsequent Cycles	
Dose level	Bolus x30 minutes IV	Continuous infusion x 4 hrs	Bolus x30 minutes IV	Continuous infusion x 4 hrs
1	30mg/m^2	30mg/m^2	30mg/m^2	50mg/m^2
2	30mg/m^2	50mg/m^2	30mg/m^2	50mg/m^2
3	30mg/m^2	50mg/m^2	40mg/m^2	60mg/m^2
4	40mg/m^2	60mg/m^2	40mg/m^2	60mg/m^2

Table 2	Cycle 1, Week 1	Cycle 1, Weeks 2–4, AND All weeks During All Subsequent Cycles

Dose level	Bolus x30 minutes IV	Continuous infusion x 4 hrs	Bolus x30 minutes IV	Continuous infusion x 4 hrs
1	30mg/m^2	30mg/m^2	30mg/m^2	50mg/m^2
1A	25mg/m^2	25mg/m^2	30mg/m^2	50mg/m^2
1B	25mg/m^2	25mg/m^2	40mg/m^2	60mg/m^2

- Administer weekly x 4 consecutive weeks, followed by 2 weeks without flavopiridol treatment for a cycle duration of 6 weeks (1 cycle).
- For cycles 2-6, administer the doses given during cycle 1, weeks 2-4 for all doses.
- Administer up to 6 cycles if no disease progression.
- Restage all positive sites of disease after every 2 cycles, except bone marrow which should be repeated after other sites have achieved a CR.
- Administer flavopiridol in the inpatient unit on cycle 1, dose 1 (bolus and infusion).
- Unless medically contraindicated, all patients will receive allopurinol 300 mg per day on cycle 1 x 5 days. All patients should receive at least 200 mL/hr 0.9% Sodium Chloride Injection for 6 hours prior to the first flavopiridol dose. Detailed required guidelines for Tumor Lysis Syndrome prevention is shown in section 4.3.
- If cycle 1, dose 1 is tolerated without TLS, subsequent doses may be administered outpatient.
- For patients who develop grade 4 neutropenia, G-CSF at a dose of 300 mcg/day SC will be instituted. Flavopiridol can be administered when the ANC is above 500. If grade 4 neutropenia persists for more than 7 days on G-CSF this will constitute a DLT and warrant a dose reduction in drug by 20%. Once G-CSF is instituted, it will be continued as prophylaxis from day 2 through day 6 of each week of treatment. G-CSF should be stopped the day prior to each flavopiridol dose.

3.2.2 Phase 2 Dosing

- <u>Dose will be the MTD from the phase I portion.</u>
- Administer weekly x 4 consecutive weeks, followed by 2 weeks without flavopiridol treatment for a cycle duration of 6 weeks (1 cycle).
- For all weeks of all cycles after cycle 1, patients will receive the dose administered on cycle 1, weeks 2-4.
- Administer up to 6 cycles if no disease progression.
- Restage all positive sites of disease after every 2 cycles, except bone marrow which should be repeated after other sites have achieved a CR.

3.2.3 Definition of Dose Limiting Toxicity:

Dose-limiting toxicity will be determined during the first cycle of treatment and is defined as:

- any grade 3 or greater non-hematologic toxicity excluding transient liver function abnormalities that resolve spontaneously within 10 days and/or transient electrolyte abnormalities that are not life-threatening, or fatigue
- grade 4 thrombocytopenia (< 25,000/mcL) persisting for 4 or more days

- grade 4 neutropenia ANC < 500/mcL persisting for 7 or more days despite the institution of G-CSF
- inability to continue with cycle 2 by 3 weeks after the end of cycle 1. Exceptions to this (i.e. accidents, family emergencies, etc) will be evaluated on a case-by-case basis.
- irreversible ≥ grade 2 non-hematologic toxicity
- irreversible grade 3 thrombocytopenia at least possibly due to flavopiridol.
- And is a drug related event with an attribution of possible, probable or definite.

Patients with compromised bone marrow function from lymphoma will not be evaluable for hematologic related DLT. Irreversible is defined as lasting > 3 weeks. All toxicity will be evaluated and scored using NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 until December 31, 2010. CTCAE version 4.0 will be utilized beginning January 1, 2011.

3.3 TREATMENT MODIFICATIONS:

Patients should have recovered toxicities that are clinically significant and attributable to flavopiridol to grade 1 or baseline before beginning another cycle. If toxicity has not resolved to this level within 3 weeks from the end of the previous cycle, flavopiridol will be discontinued.

3.3.1 Hematological Toxicity

Dose modifications of flavopiridol should be made according to the following schema:

Hematological toxicity on Day 1 of Cycle:

Platelets (at start of cycle)	**Flavopiridol Dose**
< 50,000	Delay up to 2 weeks until Plts ≥ 50,000 and reduce dose 20%.
Neutrophil count (at start of cycle)	
< 500	Start G-CSF. Administer flavopiridol when ANC is > 500. Once G-CSF is instituted, continue to administer it prophylactically for subsequent (days 2-6 each week) doses
< 500 despite G-CSF for 7 or more days	Reduce dose of flavopiridol by 20% and repeat dose only when ANC is >500

Hematological Toxicity During the Cycle:

Platelets	**Flavopiridol Dose**
< 50,000	Delay up to 2 weeks until Plts ≥ 50,000 and reduce dose 20%.
Neutrophil count	
< 500	Start G-CSF. Administer flavopiridol when ANC is > 500. Once G-CSF is instituted, continue to administer it prophylactically for subsequent (days 2-6 each week) doses
< 500 for 7 or more days despite G-CSF	Reduce dose of flavopiridol by 20% and repeat dose only when ANC is >500

If grade 4 neutropenia lasting for 7 or more days on G-CSF or grade 3 thrombocytopenia develops during treatment, hold the next dose until recovery below this toxicity and reduce dose by 20% for subsequent doses and cycles as instructed in above tables.

3.3.2 Diarrhea Toxicity

Diarrhea treatment during cycle: At first loose stool: Start loperamide 2 mg p.o. q 2 h while awake and q 4 h while sleeping. Continue around the clock until 12 h diarrhea free. If diarrhea free > 12 h, stop loperamide. Lomotil may also be given for diarrhea in addition to or as an alternative to loperamide. Please note the manufacturer's recommended maximum daily dose of loperamide is 16 mg. If grade 3 diarrhea or diarrhea is accompanied by mucus or dehydration, hold doses of flavopiridol (if applicable) and hydrate.

Diarrhea Toxicity on Previous Cycle:

Diarrhea on previous cycle	**Flavopiridol dose**
< Grade 3	No change
Grade 3 or associated with mucus or dehydration	Reduce dose by 20% on all subsequent cycles.
Grade 4	**Hold until diarrhea grade < 3 and reduce dose 20%**

Diarrhea Toxicity During the Cycle:

Diarrhea on previous week	**Flavopiridol dose**
< Grade 3	No change
Grade 3	Reduce dose by 20%. If toxicity persists for > 7 days after reduction, hold dose. .
Grade 4	**Hold until diarrhea grade < 3 and reduce dose 20%**

If grade 3 diarrhea on previous cycle, reduce dose 20% on subsequent cycles. If grade 4 diarrhea on previous cycle, hold until diarrhea < grade 3 and reduce dose 20%.

3.3.3 Deep Venous Thrombosis

If deep venous thrombosis (DVT) or pulmonary embolus (PE) or other grade 3 or 4 clotting event occurs, at the discretion of the treating investigator, therapy will be initiated with full dose Low Molecular Weight Heparin or other anticoagulants as indicated.. If recurrent DVT or PE occurs despite full and therapeutic anticoagulation, therapy with flavopiridol must be terminated and the patient will be removed from the protocol.

3.3.4 Pulmonary Reactions

Patients who develop grade 2 or 3 dyspnea with or without chest pain, temporally related to flavopiridol, will be monitored closely. Oxygen saturation and a chest x-ray should be checked. These patients may be retreated without dose reduction if the toxicity resolves completely. Patients who develop dyspnea that is temporally related to flavopiridol and is severe (at rest grade 4) or associated with tachypnea, hypoxemia, bronchospasm or pulmonary

edema/pneumonitis will be considered to have had grade 4 toxicity and will be removed from the protocol. Appropriate supportive measures including steroids and diuretics should be instituted at the discretion of the treating investigator.

3.3.5 Liver Function Tests

Liver tests, including AST, ALT and total bilirubin will be checked weekly during therapy.

- In order to initiate a cycle, liver function tests must be at grade ≤ 2.
- Within 24 hours prior to each dose, LFTs must be ≤ 2 in order for therapy to be administered.
- If liver function tests demonstrate ≥ grade 3 elevation, flavopiridol will be held until resolution to ≤ 2.
- If ≥ grade 3 elevation occurred flavopiridol will be reduced by one dose level (except for patients at dose level 1 in which case flavopiridol will be discontinued).

3.3.6 Tumor Lysis Syndrome

Tumor lysis syndrome is a term given to a group of metabolic complications that occur after treatment of neoplasms. These disorders include hyperkalemia, hyperphosphatemia, hyperuricemia, hypocalcemia and acute renal failure. Due to cases of tumor lysis syndrome encountered by Byrd et al, that have included two early fatal events associated with uncontrolled hyperkalemia, the following supportive care issues are required for patients on cycle 1 dose 1 who are participating on this trial. These serve as guidelines for management that can be modified to facilitate good patient care based upon the individual being treated.

1) Primary decisions regarding management of tumor lysis syndrome and cytokine release syndrome will be made by the principal investigator or designees. Only one patient will be initiated on flavopiridol per day to assure adequate monitoring and intervention can be provided.
2) Patients will be admitted to the in-patient service the evening prior to scheduled doses of flavopiridol, for a minimum of the first treatment (cycle 1 dose 1). Patients who do not have significant tumor lysis may receive cycle 1 doses 2-4 as well as all subsequent cycles as outpatients. This will allow inpatient monitoring of patients during the most critical window of vulnerability to tumor lysis, yet allow patients who tolerate flavopiridol to eventually be treated in the outpatient setting.
3) The Renal consult service will be made aware of all inpatient admissions for treatment on this study. Administer flavopiridol only during hours of the day when the renal service has been notified and is prepared to perform dialysis within 1 hour of notification.
4) Patients will receive IV hydration with 5% dextrose infusion with 200 mEq (200 mmol) sodium bicarbonate per Liter at 200 mL/hr for at least 10 hours prior to initiation of flavopiridol dose. This alkaline infusion will continue throughout the course of therapy and for at least 10 hours after completion of infusion.
5) Patients will begin allopurinol 300 mg orally per day at least 24 hours before cycle 1 dose 1 and continuing for at least 1 week. Rasburicase may be administered at the discretion of the treating physician but should be reserved for patients whose uric acid rises to 10 or above despite prophylaxis with allopurinol and hydration. If rasburicase is used, uric acid measurements must be drawn in a heparinized tube, placed on ice immediately and transported for rapid analysis to avoid spuriously low results.

6) Patients will receive an oral phosphate binder (Calcium Acetate 1334 mg or equivalent) the evening prior to and in the morning prior to initiation of the flavopiridol bolus.
7) Patients will have potassium levels drawn the morning within 3 hours prior to initiation of flavopiridol (with the investigator knowing the value prior to starting therapy); at the end of the bolus infusion, hourly during administration of flavopiridol; and 1, 2 and 3 hours after completion of flavopiridol dose. These tests will be performed STAT. Patients with serum potassium $\geq$ 4.0 will receive a 30 gm dose of sodium polystyrene sulfonate P.O. prior to initiation of the flavopiridol bolus.
8) Patients will continue to receive Flavopiridol as outlined in the protocol (Section 4.2).
9) Patients who develop clinical evidence of cytokine release syndrome or who have hyperkalemia requiring dialysis will receive immediate steroid therapy with an equivalent of at least 20 mg of IV dexamethasone. Cytokine syndrome is described in the CTCAE Version 3.0. It occurs during or shortly after drug infusion and generally resolves within 24 hours after completing the infusion. The signs and symptoms of cytokine syndrome include allergic reaction (including drug fever), arthralgia, bronchospasm, cough, dizziness, dyspnea, fatigue, headache, hyper- or hypotension, myalgia, nausea, pruritis, rash, rigors, chills, sweating, tachycardia, tumor pain, or urticaria.
10) Patients who develop a rise in their serum potassium to more than 4.5 mmol/L should immediately receive a 30 gm dose of sodium polystyrene sulfonate P.O..
11) Patients who develop a rise in their serum potassium to more than 5.0 mmol/L will receive a 10 unit dose of IV insulin and 50mL of 50% dextrose injection. (Prior to this intervention, it is permissible to repeat this measurement if hyperkalemia is suspected to be due to specimen hemolysis). If there is true hyperkalemia, drug administration should be discontinued.
12) Patients who develop a rise in their serum potassium to more than 5.5 will be considered for emergent intermittent or continuous hemodialysis. Prior to this intervention, it is permissible to repeat this measurement if hyperkalemia is suspected to be due to specimen hemolysis.
13) Patients who develop cytokine release (defined in 9) or who require dialysis for hyperkalemia and/or other symptoms of tumor lysis will be administered prophylactic antibiotics for Gram negative organisms (piperacillin sodium 4 gm + tazobactam sodium 0.5 gm Q8 hours or equivalent) and Gram positive organisms (vancomycin 1 gm IV Q12 hours or equivalent) until dialysis is discontinued.
14) Patients may be discharged home the day after the flavopiridol dose has been given, and their chemistry laboratory results have been reviewed by the treating physician.
15) For patients who have been initially treated for TLS, the decision about when it is safe and feasible to move them to the outpatient setting will be made by the responsible investigator. If during outpatient therapy severe tumor lysis develops requiring hemodialysis, the patient will not be retreated with flavopiridol.
16) Patients receiving flavopiridol in the outpatient clinic will begin alkaline IV hydration (similar to point 4) beginning at least 1 hour prior to initiation of the study drug. This alkaline infusion will continue throughout the course of therapy and for at least 2 hours following completion of infusion.
17) For patients who have been treated for TLS during the first cycle, a single dose of dexamethasone 20 mg PO should be administered for all further doses at the discretion of the investigator to diminish risk of cytokine release syndrome.

18) Patients will continue to take allopurinol and an oral phosphate binder, as above unless they become intolerant to it.
19) During dose 2, the potassium levels will be obtained as outlined in item 7 of section 4.3.6. Following completion of dose 2, patients will have potassium levels drawn prior to, every 2 hours during, and after completion of the flavopiridol dose in the outpatient setting. Intervention with sodium polystyrene sulfonate and/or IV insulin will be as above. Patients will not be discharged from the outpatient unit with an elevated serum potassium; if necessary, patients must be admitted overnight for observation.
20) Telemetry monitoring will be performed at the discretion of the PI or his designee.

3.3.7 **Other Clinically Significant** (determined by PI or AI) Non-Hematological Toxicity
These are adverse events that are felt to be at least possibly related to flavopiridol administration and in the opinion of the PI or AI do not have serious medical consequences (i.e. replaced easily) such as low phosphorus , low albumin or low calcium.

Symptom grade	**Management**
≤ grade 2	Treat symptomatically
≥ grade 3	Hold next dose until grade 1 or baseline and resume flavopiridol if further therapy is indicated

3.4 STUDY CALENDAR

Studies	Pre-therapy[A]	Day -1 or day 1 of each cycle	Weekly during therapy	Every second cycle	Follow-up[B]
Hx; PE; VS; PS	x	x			x
Tumor Measurement	x			x	x
CBC/diff	x[F]	x	x		x
Electrolytes, Creatinine/BUN ALT, AST, Bilirubin, LDH, Ca^{++}, Phos, Mg	x[F]	x	X		x
10 mL red top for serum storage	x	x			x
Clinical PET scan [D]	x				
CT chest/abdomen/pelvis [C]	x			x	x
Bone marrow biopsy & aspirate and peripheral blood for flow cytometry where indicated [E]	x			x[E]	
Tumor biopsy, timed blood collections and/or apheresis [G]	X				
Tumor Lysis Syndrome monitoring		X[H]			

A- Initial assessment may be performed within 4 weeks prior to starting treatment.
B- Follow-up to occur every 3-4 months until disease progression requiring initiation of non-protocol therapy.
C- Extent of CT scans may be limited to assess sites of prior disease.
D- PET scans will be performed pre-therapy if clinically indicated. PET may be obtained at the first post-treatment restaging in patients in CRu.
E- Obtain pre-treatment and after patients achieve CR by other staging.

F- These laboratory tests must be obtained within one week of initial treatment.
G- Where possible, tumor biopsies will be obtained by core needle biopsy or surgery pre-treatment. In select patients a repeat core biopsy of superficial nodes may be carried out within 24 hours from the end of the first flavopiridol infusion. Snap freeze and store tissue biopsies for translational studies at -80°C. Lymphapheresis may be performed pre-treatment in patients with leukemic MCL and may be repeated after the completion of the flavopiridol infusion. Timed blood samples of 20-40 mL each, typically pre-treatment, at 1 hour, 3hours, 6hours and 24hours may be obtained in selected patients during the first flavopiridol infusion. In patients with leukemic disease this may be repeated during the second flavopiridol infusion. Research blood collections will be limited to less than 450 mL in any 6 week period.
H- Please refer to section 4.3.6 on TLS monitoring for details of what is required.

- Pharmacokinetics**:** Collect one 6 mL sodium heparin tube at the following time points on dose 1 and 2 of cycle 1 only: 30 minutes after the start of the bolus infusion, 4.5 hours and 6 hours after the start of the bolus infusion. All PKs should be placed directly on ice and then refrigerated until specimen is picked up.
- Microarray analysis: The pretreatment lymph node biopsy, apheresis or blood sample (in the case of leukemic disease) will be used to perform all standard diagnostic tests. In addition we will extract RNA and protein for research studies. The second apheresis or the timed blood samples following the first treatment of flavopiridol will be used for research studies only. Timed blood samples may be subjected to microarray analysis to assess the effects of flavopiridol on the gene expression in normal and malignant cells.
- Proteomic analysis: In protein extracts of tumor cells pre and during therapy we aim to determine activity of key regulatory proteins such as components of the NF-kappa B signal transduction pathway, the levels of cell cycle inhibitors such as $p21^{cip}$ and $p27^{kip-1}$ and the p53 stress response. Where possible we may initiate studies that aim at characterizing changes in protein levels on a global scale.
- Tissue immunohistochemistry: Tumor tissue will be analyzed by immunohistochemistry and may include among other antigens, bcl-2, p53, MUM-1, bcl-6, MIB-1, and CD-10. Antibodies for assessment of cdk2 and cdk4 inhibition will include as many of the following as possible: anti-total Rb, anti-Rb [pT356], anti-Rb [pS249/T252], anti-Rb [pS807/811], anti-Rb [pS780], anti-Rb [pS795], anti-total $p27^{Kip1}$, and anti-$p27^{Kip1}$ [pT187]. Samples will also be analyzed for expression of cdk2, cdk4 and $p16^{INK4A}$, Ki67 and TUNEL staining. Assessment of cdk9 inhibition will be performed with anti-cyclin D1 and anti-Mcl-1, as well as anti-total RNA polymerase II, anti-RNA polymerase II [$pSer^2$] and anti-RNA polymerase II [$pSer^5$], (if RNA polymerase II antibodies can be optimized for immunohistochemistry).
- Tumor tissue may be stored for future research assays which are related to this study and do not pose an increase in patient risk.
- Serum storage: Collect one 10 mL red top tube for serum storage pre treatment and prior to each cycle.

Biopsy Procedure:
Standard techniques will be used for pre-treatment percutaneous biopsies which may include CT and / or ultrasound guided biopsy. In some cases, such biopsies may be expedited and facilitated with the use of navigation tools such as an automated laser angle selector connected to CT scan, or a standard needle guide connected to a protractor to determine which exact angle the biopsy needle will be inserted.

These guiding techniques may occur as maneuvers to facilitate the biopsy, which will take place in the usual conventional fashion, with standard, disposable, conventional spring-loaded biopsy

equipment. Such guiding techniques may add to the reliability of tissue acquisition from specific spatial coordinates of a tumor target. Accurate spatial tissue acquisition may lead to more reliable, accurate and precise tissue characterization, which in turn should be more reproducible. When performing sequential biopsies, the knowledge of sampling location within a tumor may actually help avoid multiple needle punctures or repeat procedures, as might occur when samples are obtained from necrotic regions or regions without high quality mRNA (cDNA) for microarrays or sufficient protein for proteomics analysis.

Sequential biopsy has been used routinely at the NCI as a research and prognostic tool, as well as pathway to surrogate biological markers for tumor response, prognosis, or susceptibility to specific targeted agents. Sequential biopsies are usually performed under the assumption that all tumor is created equal (mRNA and proteins) at a given time point, which is clearly an oversimplification with broad ramifications. Precision biopsy techniques might normalize some of the spatial heterogeneity inherent to tumors. Such normalization (as occurs when repeat biopsies are taken from precisely the same location of tumors) could minimize the added noise from the interpretation of already voluminous and noisy data (as in the case of the gene microarrays).

From the CT-guided biopsy, patients will receive (from two CT scans) a total of 8.0 rem to their kidneys and 5.2 rem each to their stomach, gallbladder wall and lower large intestine. All other organs will receive smaller amounts of radiation. Although each organ will receive a different dose, the amount of radiation exposure patients will receive from these procedures is equal to a uniform whole-body exposure of 1.2 rem. This calculated value is known as the "effective dose" and is used to relate the dose received by each organ to a single value. The amount of radiation received in this study is within the dose guideline established by the NIH Radiation Safety Committee for research subjects. The guideline is for an effective dose not to exceed 5 rem received per year in adults. The NIH Radiation Safety Committee has reviewed the use of radiation in adults in this research study and has approved this use as involving acceptable risk and necessary to obtain the research information desired.

In patients with leukemic disease, apheresis (lymphapheresis) will be performed for research purposes only in lieu of a tissue biopsy.

3.5 OFF STUDY CRITERIA

- Voluntary withdrawal.
- Institution of non-protocol treatment.
- Non-compliance which affects safety or endpoints of the study.
- Physician's determination that withdrawal is in the patient's best interest.

3.6 POST TREATMENT EVALUATION

Follow-up to occur every 3-4 months until disease progression that requires institution of non-protocol treatment. Adverse event data that occurs during the follow-up period that is unrelated to study treatment will not be reported. Patients who are taken off-protocol will continue to be followed for survival.

4 SUPPORTIVE CARE

4.1 TUMOR LYSIS SYNDROME

See section 4.3 in Treatment Modification Section

4.2 ANTI-EMESIS

Patients may receive prophylactic anti-emetics with ondansetron or granisetron.

4.3 NEUTROPENIA

If patients develop grade 4 neutropenia, begin treatment with G-CSF. Administer 300 mcg for all patients. Once G-CSF is instituted, continue to administer it prophylactically for subsequent (days 2-6 each week) doses on all cycles. Give G-CSF for days 2-6 each week, stopping it the day before the next dose. On dose 4, give G-CSF for days 2-6 after the dose.

5 DATA COLLECTION AND EVALUATION

5.1 DATA COLLECTION

Data will be collected and entered into the NCI C3D clinical trials database**.** Complete records must be maintained on each patient including supplementary information obtained from outside laboratories, radiology reports, or physician's records. These records will serve as the primary source material that forms the basis for the research record. The primary source documentation will assure the following:

- The patient satisfied each eligibility criterion.
- Signed informed consent was obtained prior to registration and treatment.
- Treatment was given according to protocol or any protocol violations documented and justified.
- Toxicity and response were assessed according to protocol.
- Drug accountability records were kept on each patient.

5.2 DATA REPORTING

This study will be monitored by the Clinical Data Update System (CDUS) version 3.0. Cumulative CDUS data will be submitted quarterly to CTEP by electronic means. Reports are due January 31, April 30, July 31 and October 31. Instructions for submitting data using the CDUS can be found on the CTEP web site (http://ctep.cancer.gov/reporting/cdus.html).

Effective Amendment J (version date 01/16/2015), the protocol has been completed with CTEP.

5.3 RESPONSE CRITERIA

Response criteria for lymphomas as per Cheson criteria. [18] with modifications as described below. Responses must last for at least 4 weeks off treatment.

- Complete response (CR): Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of those biochemical abnormalities (e.g. LDH) definitely assignable to the lymphoma. All lymph nodes must have regressed to normal size (≤ 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in greatest diameter must have decreased to ≤ 1 cm or by more than 75% in the sum of the products of the greatest diameters (SPD). Spleen, if considered to be enlarge before therapy, must have regressed in size and not be palpable on physical examination. The bone marrow must show no evidence of disease by

histology. Flow cytometric, molecular or cytogenetic studies will not be used to determine response.

- Complete response unconfirmed (CRu): As per CR criteria except that if a residual node is > 1.5 cm, it must have regressed by > 75% in SPD. Lymphocyte aggregates within the bone marrow must be negative for B-cell markers (e.g. L26).
- Partial response: ≥ 50% decreased in SPD of 6 largest dominant nodes or nodal masses. No increase in size of nodes, liver or spleen and no new sites of disease. Splenic and hepatic nodules must regress by ≥ 50% in the SPD. Bone marrow is irrelevant for determination of a PR.
- Definition of progressive disease (PD): Defined by at least one of the following: ≥25% increase in the sum of the products of at least two lymph nodes, appearance of new lymph nodes, ≥50% increase in the size of the liver and/or spleen as determined by measurement below the respective costal margin, appearance of new palpable hepatomegaly or splenomegaly that was not previously present, ≥50% increase in the absolute number of circulating lymphocytes.
- Definition of stable disease: (SD) will be characterized by not meeting any of the criteria outlined above.

5.4 TOXICITY CRITERIA

NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 will be utilized for AE reporting until December 31, 2010. CTCAE version 4.0 will be utilized beginning January 1, 2011.

All appropriate treatment areas should have access to a copy of the CTCAE version 3.0 and CTCAE version 4.0. A copy of the CTCAE version 3.0 and CTCAE version 4.0 can be downloaded from the CTEP web site (http://ctep.cancer.gov) . Dose limiting toxicity is defined in section 4.2.

6 STATISTICAL CONSIDERATIONS

This will be a Phase I/II study.

- The Phase I portion of the study will have three to four treatment dose levels. Three to six patients will be accrued on each of the dose levels. Enrollment will proceed on the next dose level if 0 of 3 or 1 of 6 patients experience DLT. MTD will be defined as the highest dose level in which 0-1/6 patients experience DLT.
- The Phase II portion of the study will use a Simon optimal two-stage design in each of the two histology cohorts, MCL and DLBCL. For each of these two, the trial will be designed to rule out a 20% response rate (p0=0.20) in favor of a 45% response rate (p1=0.45). The justification for this is that other regimens could be associated with a 20% response rate and the objective is to demonstrate if this agent is superior to that level. With alpha=beta=0.10, the first stage for each histology would accrue 14 patients (28 total for the first stage, but by performing response evaluation on the 6 patients treated at the MTD in the phase I portion of the study and by counting them in each phase II arm according to their histology, this would only require 22 newly enrolled patients in the first stage). If 0-

3 of the 14 in a given arm have a PR or CR, then no further patients would be accrued. If 4 or more of the 14 patients in an arm have a CR or PR, then accrual would proceed to a total of 25 patients in that arm (11 more patients beyond the 14 in the first stage). If 4 to 7 have a PR or CR, then this is insufficient activity to warrant further investigation while 8 or more responses in 25 would be adequate to justify further development. Under the null hypothesis, 20%, the probability of early termination would be 70%.

- With up to 24 patients enrolled in the phase I portion, and 50 - 6 (6 are used for response evaluation from the phase I portion)=44 new patients potentially required for the phase II portion, a total of 24+44=68 total evaluable patients may be required if both arms need to go to the second stage. To allow for a small number of non evaluable patients, the accrual ceiling will be set at 71.
- To determine if patients have responded, they will be assessed every 8 weeks. The latest time for determining a response will be after 16 weeks or 4 cycles of therapy.
- It is expected that up to 3 years may be required to accrue up to 71 total patients permitted to enroll onto this trial.
- At this time, regarding correlative studies that will be performed such as microarray and proteomic analysis, we have not as yet identified how these markers and values are modified and changed by flavopiridol therapy. Hence, we do not plan a defined statistical analysis as this research is exploratory at this time.

6.1 DATA, SAFETY AND MONITORING BOARD

All data will be collected in a timely manner and reviewed by the PI and/or study chairman for toxicity. In the event that unacceptable toxicity occurs, the IRB and IND holders will be informed and appropriate measures as outlined in the study will be taken. The monitoring of this study will be done by the PI/Study Chairman and CTEP on an ongoing manner so a DSMB will not be used.

7 HUMAN SUBJECTS PROTECTIONS

7.1 RATIONALE FOR SUBJECT SELECTION

Mantle cell lymphoma affects all races and genders but predominantly affects older patients and there is a male preponderance. This will be reflected in the distribution of cases of mantle cell lymphoma. Diffuse large B-cell lymphoma affects all races and genders. Males are more likely than females to be affected and this will be reflected in the gender distribution of our cases. This trial is directed at assessing the new drug, flavopiridol, administered by a hybrid schedule in patients with relapsed mantle cell and diffuse large B-cell lymphoma. We have selected patients with relapsed disease and have developed the trial design to recognize that some may be potentially curable and others are not. Patients under the age of 18 are excluded because inclusion of an occasional younger patient will not provide generalizable information that would justify their inclusion on this study; pediatric patients with recurrent mantle cell lymphoma or diffuse large B cell lymphoma are extremely rare and are treated on pediatric studies. Additionally, pregnant or nursing mothers are excluded because of the potential teratogenic effects of therapy.

7.2 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Patients may obtain direct benefit from treatment with flavopiridol. Results from phase I and II studies of flavopiridol show tolerable side effects. This hybrid schedule has been associated with tumor lysis syndrome in patients with CLL who have very high white cell counts but we do not anticipate this toxicity in this patient population due to very different tumor biology than CLL.

7.3 RISK/BENEFIT ANALYSIS

Patients may derive direct benefit from flavopiridol. There is in vitro evidence that demonstrates good activity of flavopiridol in patients with mantle cell lymphoma and diffuse large B-cell lymphoma. These diseases appear to have several molecular targets that are amenable to flavopiridol. The hybrid schedule of flavopiridol has been associated with excellent responses in patients with CLL and will potentially be very effective in patients with MCL and DLBCL.

7.4 CONSENT AND ASSENT

Informed written consent will be obtained in all patients on this trial. There will be no minors enrolled < 18 years old so that assent is unnecessary. The attached informed consent contains all elements required for consent. In addition, the Principal Investigator or associate investigator will provide oral consent and will be available to answer all patient questions.

8 SAFETY REPORTING REQUIREMENTS/DATA AND SAFETY MONITORING PLAN

8.1 DEFINITIONS

8.1.1 Protocol Deviation (NIH Definition)

Any change, divergence, or departure from the IRB-approved research protocol.

8.1.2 Unanticipated Problem

Any incident, experience, or outcome that:

- Is unexpected in terms of nature, severity, or frequency in relation to

  (a) the research risks that are described in the IRB-approved research protocol and informed consent document; Investigator's Brochure or other study documents, **and**

  (b) the characteristics of the subject population being studied; **AND**

- Is related or possibly related to participation in the research; **AND**
- Suggests that the research places subjects or others at a g*reater risk of harm* (including physical, psychological, economic, or social harm) than was previously known or recognized.

8.2 NCI-IRB AND CLINICAL DIRECTOR REPORTING

8.2.1 NCI-IRB and NCI CD Expedited Reporting of Unanticipated Problems and Deaths

The Protocol PI will report in the NIH Problem Form to the NCI-IRB and NCI Clinical Director:

- All deaths, except deaths due to progressive disease

- All Protocol Deviations
- All Unanticipated Problems
- All non-compliance

Reports must be received within 7 days of PI awareness via iRIS.

8.2.2 NCI-IRB Requirements for PI Reporting at Continuing Review
The protocol PI will report to the NCI-IRB:

1. A summary of all protocol deviations in a tabular format to include the date the deviation occurred, a brief description of the deviation and any corrective action.
2. A summary of any instances of non-compliance.
3. A tabular summary of the following adverse events:
   - All Grade 2 **unexpected** events that are possibly, probably or definitely related to the research;
   - All Grade 3 and 4 events that are possibly, probably or definitely related to the research;
   - All Grade 5 events regardless of attribution;
   - All Serious Events regardless of attribution.

   NOTE: Grade 1 events are not required to be reported.

8.2.3 NCI-IRB Reporting of IND Safety Reports
Only IND Safety Reports that require a sponsor recommended change to the protocol or the consent form or in the opinion of the PI increases risks to study participants will need to be reported to the NCI IRB.

8.3 CTEP ADVERSE EVENT REPORTING REQUIREMENTS

NOTE: Effective Amendment J (version date 01/16/2015), the protocol has been completed with CTEP.

ADVERSE EVENTS: LIST AND REPORTING REQUIREMENTS
Adverse event (AE) monitoring and reporting is a routine part of every clinical trial. The following list of AEs *(Section 8.2)* and the characteristics of an observed AE *(Section 8.3)* will determine whether the event requires expedited (via AdEERS) reporting **in addition** to routine (via CDUS) reporting.

8.4 THE COMPREHENSIVE ADVERSE EVENT AND POTENTIAL RISKS LIST (CAEPR)

Comprehensive Adverse Events and Potential Risks List (CAEPR) for Flavopiridol (NSC #649890)

The Comprehensive Adverse Event and Potential Risks list (CAEPR) provides a single list of reported and/or potential adverse events (AE) associated with an agent using a uniform presentation of events by body system. In addition to the comprehensive list, a subset, the Agent Specific Adverse Event List (ASAEL), appears in a separate column and is identified with ***bold*** and ***italicized*** text. This subset of AEs (ASAEL) contains events that are considered 'expected' for expedited reporting purposes only. Refer to the 'CTEP, NCI Guidelines: Adverse Event

Reporting Requirements' http://ctep.info.nih.gov/protocolDevelopment/default.htm#adverse_events_adeers for further clarification. *Frequency is provided based on 852 patients.* Below is the CAEPR for flavopiridol (Alvocidib).

[1]

Version 2.2, January 11, 2010

Adverse Events with Possible Relationship to Flavopiridol (Alvocidib) (CTCAE 4.0 Term) [n= 852]			**EXPECTED AEs FOR ADEERS REPORTING Agent Specific Adverse Event List (ASAEL)**
Likely (>20%)	**Less Likely (<=20%)**	**Rare but Serious (<3%)**	***Expected***
BLOOD AND LYMPHATIC SYSTEM DISORDERS			
Anemia			***Anemia***
	Febrile neutropenia		***Febrile neutropenia***
GASTROINTESTINAL DISORDERS			
	Abdominal pain		
Diarrhea			***Diarrhea***
Nausea			***Nausea***
Vomiting			***Vomiting***
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS			
	Chills		***Chills***
Fatigue			***Fatigue***
	Fever		***Fever***
	Pain		
IMMUNE SYSTEM DISORDERS			
	Cytokine release syndrome		***Cytokine release syndrome***
INFECTIONS AND INFESTATIONS			
	Infections and infestations - Other (Infection - Select)		***Infections and infestations - Other (Infection - Select)***
INVESTIGATIONS			
	Alanine aminotransferase increased		***Alanine aminotransferase increased***
	Aspartate aminotransferase increased		***Aspartate aminotransferase increased***
	Blood bilirubin increased		***Blood bilirubin increased***
	Lymphocyte count decreased		***Lymphocyte count decreased***
Neutrophil count decreased			***Neutrophil count decreased***
Platelet count decreased			***Platelet count decreased***
White blood cell decreased			***White blood cell decreased***
METABOLISM AND NUTRITION DISORDERS			
	Anorexia		***Anorexia***
	Hyperglycemia		***Hyperglycemia***
	Hypoalbuminemia		
	Hypophosphatemia		
	Tumor lysis syndrome		***Tumor lysis syndrome***

MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS			
	Myalgia		
NEOPLASMS BENIGN, MALIGNANT AND UNSPECIFIED (INCL CYSTS AND POLYPS)			
	Tumor pain		***Tumor pain***
NERVOUS SYSTEM DISORDERS			
	Dysgeusia		***Dysgeusia***
	Intracranial hemorrhage		
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS			
	Dyspnea		***Dyspnea***
SKIN AND SUBCUTANEOUS TISSUE DISORDERS			
	Alopecia		***Alopecia***
VASCULAR DISORDERS			
	Hypotension		***Hypotension***
	Thromboembolic event		***Thromboembolic event***

1
This table will be updated as the toxicity profile of the agent is revised. Updates will be distributed to all Principal Investigators at the time of revision. The current version can be obtained by contacting PIO@CTEP.NCI.NIH.GOV. Your name, the name of the investigator, the protocol and the agent should be included in the e-mail.

Also reported on flavopiridol (Alvocidib) trials but with the relationship to flavopiridol (Alvocidib) still undetermined:

BLOOD AND LYMPHATIC SYSTEM DISORDERS - Blood and lymphatic system disorders - Other (generalized edema); Bone marrow hypocellular
CARDIAC DISORDERS - Atrial fibrillation; Atrial flutter; Myocardial infarction; Paroxysmal atrial tachycardia; Pericardial effusion; Pericardial tamponade; Pericarditis; Sinus bradycardia; Sinus tachycardia; Supraventricular tachycardia
GASTROINTESTINAL DISORDERS - Constipation; Dry mouth; Dysphagia; Flatulence; Ileus; Mucositis oral; Rectal hemorrhage
GENERAL DISORDERS AND ADMINISTRATION SITE CONDITIONS - Non-cardiac chest pain
IMMUNE SYSTEM DISORDERS - Allergic reaction
INVESTIGATIONS - Alkaline phosphatase increased; Creatinine increased; INR increased; Weight loss
METABOLISM AND NUTRITION DISORDERS - Dehydration; Hypercalcemia; Hyperkalemia; Hyperuricemia; Hypocalcemia; Hypokalemia; Hyponatremia
MUSCULOSKELETAL AND CONNECTIVE TISSUE DISORDERS - Arthralgia; Bone pain; Generalized muscle weakness
NERVOUS SYSTEM DISORDERS - Dizziness; Dysphasia; Headache; Ischemia cerebrovascular; Neuralgia; Peripheral sensory neuropathy; Seizure; Syncope; Vasovagal reaction
PSYCHIATRIC DISORDERS - Confusion
RENAL AND URINARY DISORDERS - Acute kidney injury
RESPIRATORY, THORACIC AND MEDIASTINAL DISORDERS - Cough; Hypoxia; Pleural effusion; Respiratory failure; Respiratory, thoracic and mediastinal disorders - Other (Hemorrhage, pulmonary/upper respiratory – Select)

SKIN AND SUBCUTANEOUS TISSUE DISORDERS - Hyperhidrosis; Pruritus; Rash maculo-papular
VASCULAR DISORDERS - Phlebitis

Note: Flavopiridol (Alvocidib) in combination with other agents could cause an exacerbation of any adverse event currently known to be caused by the other agent, or the combination may result in events never previously associated with either agent.

8.5 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 3.0 will be utilized until December 31, 2010 for AE reporting. CTCAE version 4.0 will be utilized beginning January 1, 2011. All appropriate treatment areas should have access to a copy of the CTCAE version 4.0. 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 8.2 above)* for expedited reporting purposes only. 'Expected' AEs (the ASAEL) are ***bold and italicized*** in the CAEPR *(Section 8.2)*.
- **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.

8.6 EXPEDITED ADVERSE EVENT REPORTING

8.6.1 Expedited AE reporting for this study must use AdEERS (Adverse Event Expedited Reporting System), accessed via the CTEP home page (http://ctep.cancer.gov). The reporting procedures to be followed are presented in the "CTEP, NCI Guidelines: Adverse Event Reporting Requirements" which can be downloaded from the CTEP home page (http://ctep.cancer.gov). These requirements are briefly outlined in the table below *(Section 8.4.2)*.

In the rare occurrence when Internet connectivity is lost, a 24-hour notification is to be made to CTEP by telephone at 301-897-7497. Once Internet connectivity is restored, the 24-hour notification phoned in must be entered electronically into AdEERS by the original submitter at the site.

8.6.2 Expedited Reporting Guidelines – AdEERS Reporting Requirements for Adverse Events That Occur Within 30 Days[1] of the Last Dose of the Investigational Agent on Phase 1 Trials

Phase 1 Trials								
	Grade 1	Grade 2	Grade 2	Grade 3		Grade 3		Grades 4 & 5[2]
	Unexpected and Expected	Unexpected	Expected	Unexpected with Hospitali-	Unexpected without Hospitali-	Expected with Hospitali-	Expected without Hospitali-	Unexpected and Expected

				zation	zation	zation	zation	
Unrelated Unlikely	Not Required	Not Required	Not Required	10 Calendar Days	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days
Possible Probable Definite	Not Required	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days	24-Hour; 5 Calendar Days	10 Calendar Days	Not Required	24-Hour; 5 Calendar Days

[1] **Adverse events with attribution of possible, probable, or definite that occur greater than 30 days after the last dose of treatment with an agent under a CTEP IND require reporting as follows:**
AdEERS 24-hour notification followed by complete report within 5 calendar days for:
- Grade 3 unexpected events with hospitalization or prolongation of hospitalization
- Grade 4 unexpected events
- Grade 5 expected events and unexpected events

[2] Although an AdEERS 24-hour notification is not required for death clearly related to progressive disease, a full report is required as outlined in the table.

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

- Expedited AE reporting timelines defined:
  - "24 hours; 5 calendar days" – The investigator must initially report the AE via AdEERS within 24 hours of learning of the event followed by a complete AdEERS report within 5 calendar days of the initial 24-hour report.
  - "10 calendar days" - A complete AdEERS 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 via AdEERS if the event occurs following treatment with an agent under a CTEP IND.
- Use the NCI protocol number and the protocol-specific patient ID assigned during trial registration on all reports.

8.6.3 Protocol-Specific Expedited Adverse Event Reporting Exclusions

CTCAE Category	Adverse Event	Grade	Hospitalization/ Prolongation of Hospitalization	Attribution	Comments
gastrointestinal	diarrhea	3	yes	any	
blood/bone marrow	neutrophils	4	no	any	
blood/bone marrow	lymphopenia	4	no	any	
blood/bone marrow	leukocytes	4	no	any	
metabolic	phosphate, serum-low	2-4	no	any	
metabolic	Albumin, serum-low	2-4	no	any	
metabolic	calcium, serum-high	2-3	no	any	
metabolic	calcium, serum-low	2-3	no	any	

8.7 ROUTINE ADVERSE EVENT REPORTING

All Adverse Events must be reported in routine (CDUS) study data submissions. AEs reported through AdEERS must also be reported in routine study data submissions.

8.8 SECONDARY AML/MDS

AML/MDS events must be reported via AdEERS (in addition to your routine AE reporting mechanisms). In CTCAE v4.0, the event(s) may be reported as either: 1) Leukemia secondary to oncology chemotherapy, 2) Myelodysplastic syndrome, or 3) Treatment-related secondary malignancy.

9 REGULATORY CONSIDERATIONS

Cooperative Research and Development Agreement (CRADA):
"The agent(s) supplied by CTEP, DCTD, NCI used in this protocol is/are provided to the NCI under a Collaborative Agreement (CRADA, CTA, CSA) between the Pharmaceutical Company(ies) (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment and Diagnosis. Therefore, the following obligations/guidelines, in addition to the provisions in the "Intellectual Property Option to Collaborator" (http:// ctep.cancer.gov/industry) contained within the terms of award, apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used for any purpose outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and shall be maintained as such by the investigators. The protocol documents for studies utilizing investigational Agents contain confidential information and should not be shared or distributed without the permission of the NCI. If a copy of this protocol is requested by a patient or patient's family member participating on the study, the individual should sign a confidentiality agreement. A suitable model agreement can be downloaded from: http://ctep.cancer.gov.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different collaborative agreements , the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data".):
    a. NCI will provide all Collaborators with prior written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations that would tend to restrict NCI's participation in the proposed combination protocol.
    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator solely to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.
    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational Agent.

3. Clinical Trial Data and Results and Raw Data developed under a Collaborative Agreement will be made available exclusively to Collaborator(s), the NCI, and the FDA, as appropriate and unless additional disclosure is required by law or court order. Additionally, all Clinical Data and Results and Raw Data will be collected , used and disclosed consistent with all applicable federal statutes and regulations for the protection of human subjects, including, if applicable, the Standards for Privacy of Individually Identifiable Health Information set forth in 45 C.F.R. Part 164.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for Cooperative Group studies, or PI for other studies) of Collaborator's wish to contact them.
5. Any data provided to Collaborator(s) for Phase 3 studies must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial must be provided to CTEP by the Group office for Cooperative Group studies or by the principal investigator for non-Cooperative Group studies for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. Collaborator shall have the right to request that publication be delayed for up to an additional 30 days in order to ensure that Collaborator's confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts must be provided to CTEP for forwarding to Collaborator(s) for courtesy review as soon as possible and preferably at least three (3) days prior to submission, but in any case, prior to presentation at the meeting or publication in the proceedings. Press releases and other media presentations must also be forwarded to CTEP prior to release. Copies of any manuscript, abstract and/or press release/ media presentation should be sent to:

   Regulatory Affairs Branch, CTEP, DCTD, NCI
   Executive Plaza North, Suite 7111
   Bethesda, Maryland 20892
   FAX 301-402-1584
   Email: anshers@mail.nih.gov

   The Regulatory Affairs Branch will then distribute them to Collaborator(s). No publication, manuscript or other form of public disclosure shall contain any of Collaborator's confidential/ proprietary information.

10 PHARMACEUTICAL INFORMATION

10.1 CTEP-SUPPLIED AGENT

10.1.1 CTEP IND agent alvocidib (Flavopiridol), NSC #649890; IND #46211

Chemical Name: 4H-1-benzopyran-4-one, 2-(2-chloropheynl)-5, 7-dihydroxy-8-[(3S-4R)-3-hydroxy-1-methyl-4-piperidinyl]-hydrochloride

Other Names: HMR 1275, flavopiridol

CAS Registry Number: 131740-09-5

Molecular Formula: $C_{21}H_{20}ClNO_5 \bullet HCl$; M.W.: 438.29

Description: Synthetic flavone

How Supplied: Alvocidib is provided by Sanofi-Aventis Pharmaceuticals, Inc. and distributed by CTEP, NCI. It is supplied as a sterile, yellow to greenish-colored 10 mg/mL solution in flint glass vials with elastomeric closures. Each vial contains 54.5 mg of HMR 1275, which is equivalent to 50 mg of the free base, acetic acid and Water for Injection. The pH of the solution is about 3.

Preparation: The contents of the vial must be diluted prior to infusion with 0.9% Sodium Chloride Injection USP or 5% Dextrose Injection USP to final concentrations ranging from 0.09 to 1.0 mg/mL alvocodib (free base equivalent). The diluted solutions are iso-osmotic and the pH is about 3.5 to 4.1. The company currently recommends using a final concentration of 0.09 to 1 mg/mL to decrease the risk of thrombotic complications.

Storage: Store the vials at room temperature (25ºC to 30ºC) and protect from sunlight. Store diluted alvocidib solutions at room temperature and administer within 12 huors from preparation to the end of the infusion.

Stability: Shelf-life surveillance of the intact vials in on-going; intact vials have been found stable for up to 36 months at 25º C.

Dilute solutions of alvocidib are specifically compatible with silicone elastomer tubing, Becton-Dickinson filter needles, polyurethane tubing, and Hospira's Gemstar® administration sets. And they are generally compatible with IV bags composed of polyvinyl chloride (PVC) with diethylhexyl phthalate (DEHP), ethylene vinylacetate (EVA) copolymer, or low density polyethylene (LDPE) and with tubing composed of silicone elastomer, polyurethane, or PVC.

Route of Administration: Intravenous. Flavopiridol may be given peripherally or via a central venous access device.

Reported Adverse Events and Potential Risks: A list of the AEs and potential risks associated with Flavopiridol can be found in Section 8.2.

10.1.2 Agent Ordering and Agent Accountability

NCI supplied agents may be requested by the Principal Investigator (or their authorized designee). Pharmaceutical Management Branch (PMB) policy requires that agent be shipped directly to the NCI. PMB does not permit the transfer of agents between institutions (unless prior approval from PMB is obtained.) The CTEP assigned protocol number must be used for ordering all CTEP supplied investigational agents. The responsible investigator at NCI must be registered with CTEP, DCTD through an annual submission of FDA form 1572 (Statement of Investigator), Curriculum Vitae, Supplemental Investigator Data Form (IDF), and Financial Disclosure Form (FDF). If there are several participating investigators, CTEP supplied investigational agents for the study should be ordered under the name of one lead investigator at that institution.

Agent may be requested by completing a Clinical Drug Request (NIH-986) and mailing it to the Drug Management and Authorization Section, PMB, DCTD, NCI, 9000 Rockville Pike, EPN

Room 7149, Bethesda, MD 20892-7422 or faxing it to (301) 480-4612. For questions call (301) 496-5725.

Agent Inventory Records - The investigator, or a responsible party designated by the investigator, must maintain a careful record of the inventory and disposition of all agents received from DCTD using the NCI Drug Accountability Record (DAR) Form. (See the NCI Investigator's Handbook for Procedures for Drug Accountability and Storage.)

10.2 OTHER AGENT(S)

10.2.1 Allopurinol

Supply: Commercially available.

Pharmacology: Allopurinol reduces the production of uric acid by inhibiting xanthine oxidase, the enzyme responsible for conversion of hypoxanthine to xanthine and of xanthine to uric acid, resulting in reductions in plasma and urinary concentrations of uric acid.

Product description: Allopurinol is available in 100mg tablets and 300mg tablets. Allopurinol sodium is also available as a powder for injection for intravenous administration. It is not anticipated that intravenous allopurinol will be utilized for the purpose of this protocol.

Storage and Stability: Oral tablets should be stored at 15º to 25ºC (59º to 77ºF).

Route of administration: Oral

Dose: 300 mg given orally once daily if estimated creatinine clearance is greater than 20 mL/min. Follow product labeling guidelines for dose adjustment if estimated creatinine clearance is less than or equal to 20 mL/min.

Toxicities: Allopurinol hypersensitivity or allergic reactions manifest by varying kinds of skin rash in association with fever, chills, leukopenia or leukocytosis, eosinophilia, arthralgia and pruritus. Dermatological complications related to allopurinol therapy are common and may occur in up to 10% to 15% of cases particularly if allopurinol is used concurrently with ampicillin or amoxicillin. The most common side effect of allopurinol is a MACULOPAPULAR RASH, often preceded by pruritus, an important warning symptom and an indication to discontinue the drug to avoid progression to more severe reactions (Prod Info Zyloprim(R), 2000). Allopurinol has produced hepatotoxic effects ranging from an alteration in liver function tests to hepatitis, which may be a part of a generalized hypersensitivity reaction.

11 REFERENCES

1. Carlson, B.A., et al., Flavopiridol induces G1 arrest with inhibition of cyclin-dependent kinase (CDK) 2 and CDK4 in human breast carcinoma cells. Cancer Res, 1996. 56(13): p. 2973-8.
2. Kaur, G., et al., Growth inhibition with reversible cell cycle arrest of carcinoma cells by flavone L86-8275. J Natl Cancer Inst, 1992. 84(22): p. 1736-40.
3. Worland, P.J., et al., Alteration of the phosphorylation state of p34cdc2 kinase by the flavone L86-8275 in breast carcinoma cells. Correlation with decreased H1 kinase activity. Biochem Pharmacol, 1993. 46(10): p. 1831-40.

4. Lam, L.T., et al., Genomic-scale measurement of mRNA turnover and the mechanisms of action of the anti-cancer drug flavopiridol. Genome Biol, 2001. 2(10): p. RESEARCH0041.
5. Takada, Y. and B.B. Aggarwal, Flavopiridol inhibits NF-kappaB activation induced by various carcinogens and inflammatory agents through inhibition of IkappaBalpha kinase and p65 phosphorylation: abrogation of cyclin D1, cyclooxygenase-2, and matrix metalloprotease-9. J Biol Chem, 2004. 279(6): p. 4750-9.
6. Senderowicz, A.M., et al., Phase I trial of continuous infusion flavopiridol, a novel cyclin-dependent kinase inhibitor, in patients with refractory neoplasms. J Clin Oncol, 1998. 16(9): p. 2986-99.
7. Arguello, F., et al., Flavopiridol induces apoptosis of normal lymphoid cells, causes immunosuppression, and has potent antitumor activity In vivo against human leukemia and lymphoma xenografts. Blood, 1998. 91(7): p. 2482-90.
8. Byrd, J.C., et al., Treatment of relapsed chronic lymphocytic leukemia by 72-hour continuous infusion or 1-hour bolus infusion of flavopiridol: results from Cancer and Leukemia Group B study 19805. Clin Cancer Res, 2005. 11(11): p. 4176-81.
9. Kouroukis, C.T., et al., Flavopiridol in untreated or relapsed mantle-cell lymphoma: results of a phase II study of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol, 2003. 21(9): p. 1740-5.
10. Lin, T.S., et al., Seventy-two hour continuous infusion flavopiridol in relapsed and refractory mantle cell lymphoma. Leuk Lymphoma, 2002. 43(4): p. 793-7.
11. Tan, A.R., et al., Phase I clinical and pharmacokinetic study of flavopiridol administered as a daily 1-hour infusion in patients with advanced neoplasms. J Clin Oncol, 2002. 20(19): p. 4074-82.
12. Bible, K.C. and S.H. Kaufmann, Flavopiridol: a cytotoxic flavone that induces cell death in noncycling A549 human lung carcinoma cells. Cancer Res, 1996. 56(21): p. 4856-61.
13. Schwartz, G., et al., Flavopiridol enhances the biologic effects of paclitaxel: A phase I trial in patients with advanced solid tumors. Proc Am Soc Clin Oncol 17: 188, 1998: 725, 1998.
14. Thomas, J.P., et al., Phase I clinical and pharmacokinetic trial of the cyclin-dependent kinase inhibitor flavopiridol. Cancer Chemother Pharmacol, 2002. 50(6): p. 465-72.
15. Zhai, S., et al., Flavopiridol, a novel cyclin-dependent kinase inhibitor, in clinical development. Ann Pharmacother, 2002. 36(5): p. 905-11.
16. Schwartz, G.K., et al., Phase I study of the cyclin-dependent kinase inhibitor flavopiridol in combination with paclitaxel in patients with advanced solid tumors. J Clin Oncol, 2002. 20(8): p. 2157-70.
17. Byrd, J.C., et al., Flavopiridol administered using a pharmacologically derived schedule is associated with marked clinical efficacy in refractory, genetically high-risk chronic lymphocytic leukemia. Blood, 2006.
18. Cheson, B.D., et al., Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. J Clin Oncol, 1999. 17(4): p. 1244.

12 APPENDIX A: Specimen Collection and Storage

All specimens obtained in the protocol are used as defined in the protocol. Any specimens that are remaining at the completion of the protocol will be stored in the conditions described below for an indefinite amount of time. The PI will report any loss or destruction of samples to the NCI IRB as soon as he is made aware of such loss.

12.1 PHARMACOKINETIC SAMPLES

All data associated with samples sent to the Blood Processing Core (BPC) under the direction of Dr. Figg is entered into the Clinical Pharmacology Program's LABrador database (aka LabSamples or, formerly, "Patient Sample Database Management System" (PSDMS)). This is a secure program that can only be accessed by authorized users in Dr. Figg's lab. LABrador creates a unique barcode ID for every sample and sample box which cannot be traced back to patients without LABrador access.

The data recorded for each sample includes the patient ID, name, trial name/protocol number, time drawn, cycle time point, dose, material type, as well as box and freezer location. There are patient demographics that can be obtained to correlate with the samples through the system. For each samples, there are notice associate with processing method (delay in sample processing, storage conditions on the ward, etc.). Bar-coded samples are stored in bar-coded boxes in a locked freezer at either -20 or -85°C according to stability requirements. These freezers are located onsite in Dr. Figg's lab and offsite at NCI Frederick Central Repository Services in Frederick, MD. Samples will be stored until requested by the researcher assigned to the protocol (however, those requests must come from a member of Dr. Figg's laboratory with LABrador access/clearance). All requests are monitored and tracked in the system. All researchers are required to sign a form stating that the samples are only to be used for research purposes associated with this trial (as per the IRB approved protocol – that protocol is stored in the LABrador system) and that any unused samples must be returned to Dr. Figg's laboratory.

12.2 PROTOCOL COMPLETION/SAMPLE DESTRUCTION

Once primary research objectives for the protocol are achieved, intramural researchers can request access to remaining samples providing they have an IRB approved protocol and patient consent.

The PI will report destroyed samples to the IRB if samples become unsalvageable because of environmental factors (ex. broken freezer or lack of dry ice in a shipping container) or if a patient withdraws consent. Samples will also be reported as lost if they are lost in transit between facilities or misplaced by a researcher. Dr. Figg's laboratory will report any freezer problems, lost samples or other problems associated with samples to the IRB, the NCI Clinical Director, and the office of the CCR, NCI.

12.3 PROCEDURES FOR STORED SERUM SPECIMENS

The Clinical Support Laboratory, Leidos Biomedical Research, Inc.-Frederick, processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. All laboratory personnel with access to patient information annually complete the NIH online course in Protection of Human Subjects. The laboratory is CLIA certified for CD4 immunophenotyping and all laboratory areas operate under a Quality Assurance Plan with documented Standard Operating

Procedures that are reviewed annually. Laboratory personnel are assessed for competency prior to being permitted to work with patient samples. Efforts to ensure protection of patient information include:

- The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.
- An electronic database is used to store information related to patient samples processed by the laboratory.
    - The database resides on a dedicated program server that is kept in a central, locked computer facility.
    - The facility is supported by two IT specialists who maintain up to date security features including virus and firewall protection.
    - Program access is limited to specified computers as designated by the laboratory director. Each of these computers has a password restricted login screen.
    - The database sample entry program itself is accessed through a password protected entry screen.
    - The database program has different levels of access approval to limit unauthorized changes to specimen records and the program maintains a sample history.
- Upon specimen receipt each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID.
- Beginning October 1, 2006 inventory information will be stored at the vial level and each vial will be labeled with both a sample ID and a vial sequence number.
- Vial labels do not contain any personal identifier information.
- Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long term storage. These facilities are operated by Fisher Bioservices, Inc. under subcontract to Leidos Biomedical Research, Inc.-Frederick.
- Access to stored clinical samples is restricted. Investigators establish sample collections under “Source Codes” and the investigator responsible for the collections, typically the protocol Principal Investigator, specifies who has access to the collection. Beginning October 1, 2006 specific permissions will be required to view, input or withdraw samples from a collection. Prior to that date sample input was not restricted and restrictions were limited to specimen withdrawal.
- Sample withdrawal requests submitted to approved laboratory staff by anyone other than the repository source code owner are submitted to the source code owner for approval. Beginning October 1, 2006 the repository facility will also notify the Source Code holder of any submitted requests for sample withdrawal.
- It is the responsibility of the Source Code holder (generally the NCI Principal Investigator) to ensure that samples requested and approved for withdrawal are being used in a manner consistent with IRB approval.

  - The Clinical Support Laboratory does perform testing services that may be requested by clinical investigators including, but not limited to, immunophenotyping by flow cytometry and cytokine testing using ELISA or multiplex platforms.
- When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement is in place that covers the specimen transfer. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process.
- The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that a Material Transfer Agreement has been executed prior to requesting the laboratory to ship samples outside of the NIH.

12.4 PROCEDURES FOR PERIPHERAL BLOOD CELLS

1. Orders for tumor biopsies, research blood samples and lymphapheresis collections should be placed in CRIS (Clinical Research Information System, Clinical Research Center, NIH, Bethesda, MD)
2. Tumor biopsies will be submitted in native condition to the Department of Pathology, CRC, NIH and handled according to routine procedures. Material released for research studies will be documented on form NIH 2803-1. Initial processing of samples for research will depend on the size of the tumor biopsy. For core biopsies the research sample will typically consist of 2 cores in a microcentrifuge vial snap frozen on dry ice. Surgical lymph node biopsies may in addition be processed for single cell suspension, additional vials of snap frozen tissue and OCT embedded tissue.
3. Lymphapheresis is performed in the Department of Transfusion Medicine, and blood will be collected in the phlebotomy suite, on a clinical ward, or in an outpatient clinic of the CRC, NIH. Samples will be transferred to the research laboratory at room temperature. Cells will be separated by Ficoll density gradient centrifugation and only mononuclear cells will be harvested, processed, analyzed, and stored.
4. Tumor and normal blood cells may be viably frozen, typically at concentrations of 20-100x10^6/mL in FCS with 10% DMSO using a temperature controlled freezing process to optimize sample viability. Samples will be transferred to Nitrogen tanks for long term storage.
5. Tumor and normal blood cells can be further processed. Additional purification may be carried out by selection with magnetic beads binding to appropriate surface molecules, typically CD19. For analysis cells may be lysed to obtain RNA (using Qiagen manufactured kits are similar) or proteins (salt and/or triton containing buffers with addition of protease and phosphatase inhibitors). Integrity of RNA is monitored by gel electrophoresis and concentration of RNA or protein is measured spectrophotometrically.
6. Research sample inventory and storage. All research samples are assigned a unique number and cataloged. Viably frozen cells are stored in a temperature controlled, alarm secured Nitrogen tank. Tumor biopsies and processed biologic material (RNA, protein) is stored at -80C in a temperature controlled, alarm secured -80C freezer.

12.5 PROCEDURES FOR LYMPH NODE BIOPSIES SENT TO DANA FARBER CANCER INSTITUTE

Procedures. Lymph node biopsies will be obtained within 1 week of the first Flavopiridol dose and at the conclusion of a Flavopiridol infusion. Biopsies will be fixed in formalin and either shipped on the day of acquisition to the Dana-Farber/Harvard Cancer Center Pathology Core or processed at NIH. Formalin-fixed, paraffin-embedded 5-μM sections will be mounted on glass slides, and subjected to immunohistochemistry with an automated stainer (BioGenex, San Roman, CA). Antibodies for assessment of cdk2 and cdk4 inhibition will include as many of the following as possible: anti-total Rb, anti-Rb [pT356], anti-Rb [pS249/T252], anti-Rb [pS807/811], anti-Rb [pS780], anti-Rb [pS795], anti-total $p27^{Kip1}$, and anti-$p27^{Kip1}$ [pT187]. Samples will also be analyzed for expression of cdk2, cdk4 and $p16^{INK4A}$, Ki67 and TUNEL staining. Assessment of cdk9 inhibition will be performed with anti-cyclin D1 and anti-Mcl-1, as well as anti-total RNA polymerase II, anti-RNA polymerase II [$pSer^2$] and anti-RNA polymerase II [$pSer^5$], (if RNA polymerase II antibodies can be optimized for immunohistochemistry).

Scoring of immunohistochemical sections will be performed by Dr. Lucian Chirieac (Brigham and Women's Hospital, Department of Pathology). A minimum of 200 cells will be scored for nuclear staining and will be graded as 0, 1+ or 2+. The percentage of all positive cells and strongly positive cells will be compared pre- and post treatment, so that for each pair of specimens, the percent change from baseline of these parameters will be calculated.

We will also examine the association between objective response and stable disease and baseline levels of cyclin D1 and $p16^{INK4A}$ in tumor biopsies, as well as microarray analysis performed at the NCI, to assess the relationship between the initial proliferative signature and clinical outcome after Flavopiridol treatment.

Statistical considerations. The major objectives will be to demonstrate that Flavopiridol has inhibited cdk targets within lymphoma cells and to determine whether the modulation of cdk targets correlates with response. Statistical significance between levels of biomarkers pre- and post-treatment will be assessed by the Wilcoxon signed rank test. The relationship between changes in phospho-Rb, $p27^{Kip1}$, phospho-RNA polymerase II, cyclin D1, Mcl-1 and Ki67 and radiographic tumor response will be assessed using rank-based correlation methods for pairs of quantitative endpoints, and by the Wilcoxon rank-sum test for the association between quantitative endpoints and radiographic response.

Samples were sent to:
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