STUDY PROTOCOL

Protocol Number X05260

A Phase II, Open-Label Study of Bortezomib(Velcade®), Cladribine, and Rituximab (VCR) in Advanced, Newly Diagnosed and Relapsed/Refractory Mantle Cell and Indolent Lymphomas

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Investigator & Study Center:

Principal Investigator:
Daniel Persky, MD
University of Arizona Cancer Center
1515 N. Campbell Ave
Tucson, AZ 85724-5024
Telephone: (520) 626-8908

Co-PI:
Faiz Anwer, MD
University of Arizona Cancer Center
1515 N. Campbell Ave
Tucson, AZ 85724-5024
Telephone: (520) 626-0662

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PROTOCOL SUMMARY

Title: A Phase II, Open-Label Study of Bortezomib(Velcade®), Cladribine, and Rituximab (VCR) in Advanced, Newly Diagnosed and Relapsed/Refractory Mantle Cell and Indolent Lymphomas

Objectives

The primary objective of this study is to:

- Determine 2-year PFS in patients with mantle cell, marginal zone, lymphoplasmacytic, small lymphocytic, and relapsed follicular lymphomas

The secondary objectives of this study are to:

- Determine 2-year OS;
- Determine CR and ORR;
- Describe long and short-term toxicity;
- Conduct correlative studies:
    - Determine prognostic importance of Aurora kinase A in lymphoma treated with VCR;
    - Determine the cytokine profiles of each lymphoma subtype and how they change with VCR;
    - Evaluate the prognostic importance of major oncogenic pathways in lymphoma treated with VCR using tissue microarray (TMA)

Patient population

Eligible patients must have one of the following histologies: mantle cell lymphoma, lymphoplasmacytic lymphoma (Waldenstrom's macroglobulinemia), marginal zone lymphoma, small lymphocytic lymphoma, or relapsed follicular lymphoma. They may or may not have been treated for their lymphoma.

Specific inclusion and exclusion criteria are detailed in section 3.2.

Number of patients

39

Study design and methodology

Prospective open-label phase II trial

Treatments administered

- Rituximab 375 mg/m2 IV day1
- Cladribine 4 mg/m2 IV over 2 hours days 1-5
- Bortezomib 1.3 mg/m2 IV days 1 and 4
- Repeat every 28 days for a maximum of 6 cycles

Efficacy data collected

The following evaluations will be conducted to assess the efficacy of VELCADE-Cladribine-Rituximab:

- Assessment of progression-free survival
- Assessment of overall survival
- Assessment of response according to the International Workshop Group response criteria (Cheson

et al., 1999). If PET scan is used for staging, revised response criteria should be used (Cheson et al., 2007). For Waldenstrom's Macroglobulinemia, response assessment should additionally be performed according to the Third International Workshop on Waldenstrom's Macroglobulinemia (Treon et al., 2006).

Pharmacokinetic/Pharmacodynamic/Pharmacogenomic/Correlative studies collected (optional)

The following studies will be conducted to assess the pharmacokinetics/pharmacodynamics/pharmacogenomics of VELCADE-Cladribine-Rituximab:

- None

The following studies will be performed at Millennium:

- None

The following correlative studies will be performed at the Arizona Cancer Center:

- Serum cytokine profile of >175 serum cytokines utilizing an antibody platform with normal controls, to evaluate major cytokines in mantle cell lymphoma, lymphoplasmacytic lymphoma (Waldenstrom's macroglobulinemia), small lymphocytic lymphoma, and follicular lymphoma. The changes in cytokine levels will be compared between lymphoma subtypes and between responders and non-responders.
- Tissue microarray (TMA) to evaluate major oncogenic pathways in lymphoma. Separate consent will be requested for an optional biopsy to assess for post-therapy changes at cycle 2, day 1. In particular, the importance of Aurora kinase A will be evaluated.

Safety data collected

The following evaluations will be conducted to assess the safety of VELCADE-Cladribine-Rituximab:

- Toxicity assessment using CTCAE, version 3.0

Statistical procedures

The primary outcome measure of this Phase II trial is progression-free survival (PFS) at 2 years. This will be estimated for the cohort and presented along with appropriate confidence intervals. The Kaplan-Meier product-limit method will be used to estimate progression-free survival in the presence of censoring. A 2-year PFS of 70% or more would be considered promising, while a 2-year PFS of less than 50% would be disappointing. Thus we will test whether we can reject the null hypothesis of 50% or less versus the desired alternative rate of 70% or more. Based on an exact test for a single binomial proportion at the one-sided 0.10 significance level, a sample size of 39 patients would provide a power of 90% (i.e., maximum false negative rate of 10% and false positive rate of 10%); this calculation assumes that all patients would be followed until progression or for at least 2 years. All patients entered into the trial who receive at least one dose of treatment will be included in the analysis. With 39 patients, there will be greater than a 90% probability of observing any specific type of adverse event whose true incidence rate is 6% or higher.

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ABBREVIATIONS LIST

Abbreviation	Definition
°C	degrees Celsius
µM	micromolar
20S	20S proteasome subunit
AE	adverse event
ANC	absolute neutrophil count
Bc1-2	B-cell lymphoma-2; a gene that inhibits apoptosis
BSA	body surface area
CAM	cell adhesion molecules
cm	centimeter
CHOP	Cyclophosphamide, Doxorubicin, Vincristine, Prednisone
CR	Complete Response
CTCAE	(NCI) Common Terminology Criteria for Adverse Events
CTEP	Cancer Therapy Evaluation Program
CVP	Cyclophosphamide, Vincristine, Prednisone
dL	deciliter
DLT	Dose Limiting Toxicity
DNA	deoxyribonucleic acid
FDA	Food and Drug Administration
FL	Follicular Lymphoma
GCP	Good Clinical Practice
GLP	Good Laboratory Practice
ht	height
IκB	I kappa B kinase; cytokine response kinase that activates transcription factor NF-kappa b at serine 32 and 36
ICAM-1	intercellular adhesion molecule 1
ICH	International Conference on Harmonisation
IEC	Independent Ethics Committee
IND	Investigational New Drug
IRB	Institutional Review Board
IV	intravenous
IκBα	I kappa B alpha-associated protein kinase
kg	kilogram
Ki	inhibitory constant
lbs	pounds
LDH	Lactate Dehydrogenase
m^2	square meters

Abbreviation	Definition
MCL	Mantle Cell Lymphoma
mg	milligram
min	minute
mL	milliliter
mm^3	cubic millimeters
mmol	millimole
MTD	Maximum Tolerated Dose
NCI	National Cancer Institute
NF-κB	nuclear factor-κB
NHL	Non-Hodgkin's Lymphoma
ng	nanogram
nM	nanomole
ORR	Overall Response Rate
p21	p21(ras) farnesyl-protein transferase
p27	cyclin-dependent kinase inhibitor
p53	tumor suppressor protein with molecular weight of 53 kDa
PR	Partial Response
PS	Performance Status
SAE	serious adverse event
TMA	Tissue Microarray
US	United States
USP	United States Pharmacopeia
VCAM-1	vascular cell adhesion molecule 1
w/w	weight-to-weight ratio
WM	Waldenstrom's macroglobulinemia
wt	weight

1 INTRODUCTION AND STUDY RATIONALE

1.1 Overview of the Disease

Mantle cell lymphoma accounts for 6% of all non-Hodgkin's lymphomas (NHL), diagnosed in approximately 3800 patients each year in the United States. It is an incurable lymphoma with a median overall survival of 3-5 years, but with great variability between the patients. Mantle cell lymphoma is characterized by initial sensitivity to both chemotherapy and radiation but also by an invariable relapse and eventual resistance to treatment. There is no established standard of care. Currently used regimens such as R-hyper-CVAD/R-M-A and autologous stem cell transplantation (ASCT) in first remission are notable for their toxicity. VELCADE is approved as a single agent for relapsed mantle cell lymphoma and is being actively investigated in combination therapy as part of initial therapy, which is one of the main objectives of this trial as well.

Indolent lymphomas such as follicular, lymphoplasmacytic, marginal zone, and small lymphocytic lymphomas represent approximately a third of all NHL diagnosed in the United States, or about 21,000 patients a year. They are incurable, with median overall survival of 8-10 years that varies greatly between the patients. Current standard of care is watchful waiting until certain treatment criteria are met. Once decision to treat is made, there is no standard treatment regimen. Several combination therapy regimens are considered, incorporating rituximab, an anti-CD20 monoclonal antibody, in combination with an alkylator, with an anthracycline (+/- an alkylator), or with a purine analog (+/- an alkylator). Combinations including VELCADE are also investigated. This protocol will investigate combination of VELCADE with rituximab and a purine analog cladribine.

The median age at diagnosis of both mantle cell and indolent lymphomas listed above is approximately 65 years, which precludes many patients from receiving intensive chemotherapy. Therefore, it is particularly important to develop a non-toxic regimen to induce durable remissions.

1.2 VELCADE (bortezomib) for Injection

1.2.1 Scientific Background

VELCADE™ (bortezomib) for Injection is a small molecule proteasome inhibitor developed by Millennium Pharmaceuticals, Inc., (Millennium) as a novel agent to treat human malignancies. VELCADE is currently approved by the United States Food and Drug Administration (US FDA) for the treatment of patients with multiple myeloma (MM). It is also indicated for the treatment of patients with mantle cell lymphoma (MCL) who have received at least 1 prior therapy. In the European Union (EU), VELCADE in combination with melphalan and prednisone is indicated for the treatment of patients with previously untreated MM who are not eligible for high-dose chemotherapy with bone marrow transplant. VELCADE is indicated as monotherapy for the treatment of progressive MM in patients who have received at least 1 prior therapy and who have already undergone or are unsuitable for bone marrow transplantation.

By inhibiting a single molecular target, the proteasome, bortezomib affects multiple signaling pathways. The anti-neoplastic effect of bortezomib likely involves several distinct mechanisms, including inhibition of cell growth and survival pathways, induction of apoptosis, and inhibition of expression of genes that control cellular adhesion, migration and angiogenesis. Thus, the mechanisms by which bortezomib elicits its antitumor activity may vary among tumor types, and the extent to which each affected pathway is critical to the inhibition of tumor growth could also differ. Bortezomib has a novel pattern of cytotoxicity in National Cancer Institute (NCI) in vitro and in vivo assays (Adams et al., 1999). In addition, bortezomib has cytotoxic activity in a variety of xenograft tumor models, both as a single agent and in combination with chemotherapy and radiation (Steiner et al., 2001; Teicher et al., 1999; Cusack et al., 2001; LeBlanc et al., 2002; Pink et al., 2002). Notably, bortezomib induces apoptosis in cells that over express bcl-2, a genetic trait that confers unregulated growth and resistance to conventional chemotherapeutics (McConkey et al., 1999).

Bortezomib is thought to be efficacious in multiple myeloma via its inhibition of nuclear factor κB (NF-κB) activation, its attenuation of interleukin-6 (IL-6)-mediated cell growth, a direct apoptotic effect, and possibly anti-angiogenic and other effects (Hideshima et al., 2001).

The mechanisms of action leading up to apoptosis have been more clearly defined and include initiation of the unfolded protein response and direct/indirect effects on various molecular targets including cell cycle control proteins p27 and p21, cyclins, signal transduction molecules, transcription factors c-jun and HIF1-α, tumor suppressor protein p53, angiogenesis factors, and many others. Bortezomib is thought to be efficacious in mantle cell lymphoma by inhibiting p27 degradation, leading to cell cycle arrest and apoptosis. Increased proteasome degradation of p27 has been associated with decreased survival in mantle cell lymphoma (Chiarle et al, 2000). The mechanism of activity in other lymphoma subtype has not been elucidated, but may be related to the inhibition of nuclear factor κB (NF-κB) activation (O'Connor et al, 2005).

1.2.2 **Nonclinical Pharmacology**

Pharmacokinetic (PK) and pharmacodynamic studies were conducted in the rat and cynomolgus monkey. Upon intravenous (IV) bolus administration, bortezomib displays a rapid distribution phase ($t_{½}\alpha$ <10 minutes) followed by a longer elimination phase ($t_{½}\beta$ 5–15 hours). Bortezomib has a large volume of distribution (range 5–50 L/kg). The plasma PK profile is well described by a 2-compartment model.

The pharmacodynamic action of bortezomib is well established and can be measured through an ex vivo assay (20S proteasome activity) (Lightcap et al., 2000). This assay was used to determine the duration of drug effect in lieu of the PK data in the early preclinical toxicology studies as well as to set a guide for dose escalation in humans. Following dosing with bortezomib in the rat and cynomolgus monkey, proteasome inhibition in peripheral blood had a half-life less than 24 hours, with proteasome activity returning to pretreatment baseline within 24 hours in monkey and within 48 to 72 hours in rat after a single dose of bortezomib. Further, intermittent but high inhibition (>70%) of proteasome activity was better tolerated than sustained inhibition. Thus, a twice-

weekly clinical dosing regimen was chosen in order to allow return of proteasome activity towards baseline between dose administrations.

1.2.3 **Nonclinical Toxicity**

Single-dose IV toxicity studies were conducted with bortezomib in the mouse, rat, dog, and monkey to establish the single-dose maximum tolerated dose (MTD). The MTDs were 0.25 mg/kg (1.5 mg/m^2) and 0.067 mg/kg (0.8 mg/m^2) in the 2 most sensitive species, rat and monkey, respectively.

Repeat-dose multi-cycle toxicity studies of 3 and 6 months in the rat and 9 months in the monkey, each with 8-week recovery periods, were conducted to characterize the chronic toxicity of bortezomib when administered by the clinical route and regimen of administration. The MTD in the 6-month rat study was 0.10 mg/kg (0.6 mg/m^2) and the key target organs were the gastrointestinal (GI) tract, hematopoietic and lymphoid systems. The MTD in the 9-month monkey study was 0.05 mg/kg (0.6 mg/m^2) and the key target organs were the GI tract, hematopoietic and lymphoid systems, peripheral nervous system, and kidney. Full or partial reversibility was observed for each of the toxicities described to date.

In general, the nature of the toxicity of bortezomib is similar across species, and target organs of toxicity in animals have been largely predictive of human toxicity. The toxicity of bortezomib in animals is characterized by a steep dose-response with mortality seen at dosages above the MTD. The cause of death at acutely lethal dosages is considered to be related to indirect cardiovascular (CV) effects of hypotension and vascular changes with secondary bradycardia and the cause of death in long-term studies has been attributed to GI or hematologic toxicity. The pharmacologic effects of bortezomib on the CV system have been extensively characterized and have demonstrated that indirect effects on CV function occur only at acutely lethal dosages and are abrogated by routine supportive care.

Additional detailed information regarding the nonclinical pharmacology and toxicology of bortezomib may be found in the most recent Investigator's Brochure

1.2.4 **Clinical Pharmacokinetics and Pharmacodynamics**

The clinical pharmacology characterization of bortezomib has been determined from phase 1 studies in subjects with solid tumors and hematological malignancies, and confirmed in phase 2 studies in subjects with multiple myeloma.

Bortezomib demonstrates multi-compartmental pharmacokinetics. Following intravenous administration of 1.0 mg/m^2 and 1.3 mg/m^2 dose, the mean first-dose maximum observed plasma concentrations of bortezomib were 57 and 112 ng/mL, respectively in 11 patients with multiple myeloma and creatinine clearance values >50 mL/min participating in a pharmacokinetics study. In subsequent doses, mean maximum observed plasma concentrations ranged from 67 to 106 ng/mL for the 1.0 mg/m^2 dose and 89 to 120 ng/mL for the 1.3 mg/m^2 dose. The mean elimination half-life of bortezomib upon multiple dosing ranged from 40 to 193 hours. Bortezomib is eliminated more rapidly following the first dose. Mean Total Body Clearances were 102 and 112

L/h following the first dose for doses of 1.0 mg/m^2 and 1.3 mg/m^2, respectively, and ranged from 15 to 32 L/h following subsequent doses for doses of 1.0 and 1.3 mg/m^2, respectively. Clinical experience has shown that the change in clearance does not result in overt toxicity from accumulation in this multidose regimen in humans.

In subjects with advanced malignancies, the maximum pharmacodynamic effect (inhibition of 20S activity) occurred within 1-hour post dose. At the therapeutic dose of 1.3 mg/m^2 in subjects with multiple myeloma, the mean proteasome inhibition at 1-hour post dose was approximately 61%.
The time course of proteasome inhibition in subjects is characterized by maximum inhibition observed within the first hour after administration, followed by partial recovery of proteasome activity over the next 6 to 24 hours to within 50% of the pretreatment activity. On the Day 1, 4, 8, and 11 schedule, variable (10%–30%) levels of proteasome inhibition have been observed at next scheduled dosing. In theory, this advantage allows cells to recover proteasome activity for normal cellular housekeeping functions between doses.

The relationship between bortezomib plasma concentrations and proteasome inhibition can be described by a maximum effect (E_{max}) model. The E_{max} curve is initially very steep, with small changes in plasma bortezomib concentration over the range of 0.5 to 2.0 ng/mL relating to large increases in the percent inhibition (0–60%). After that, a plateau occurs where marginal increases of proteasome inhibition are observed in spite of large changes in plasma bortezomib concentrations.

1.2.5 **Clinical Experience**

It is estimated that as of June 2011, more than 300,000 patients have been treated with VELCADE, including patients treated through Millennium-sponsored clinical trials, Investigator-Initiated Studies, the US NCI Cancer Therapy Evaluation Program (CTEP), and with commercially available drug. VELCADE has been commercially available since 13 May 2003.

The overall goal of the Millennium phase 1 program was to determine the MTD and dose-limiting toxicity (DLT) of VELCADE in a number of therapeutic settings involving subjects with various advanced malignancies. In a Phase I trial in patients with refractory hematologic malignancies, the MTD for a twice weekly dosing for 4 weeks of a 42 day cycle was 1.04 mg/m^2/dose, with DLTs of thrombocytopenia, hyponatremia, hypokalemia, fatigue, and malaise (Orlowski et al., 2002). The toxicity was greatest during the third and fourth weeks of therapy. In the 3-week schedule of VELCADE monotherapy (4 doses, given on Days 1, 4, 8, and 11 of a 21-day treatment cycle), the DLT occurred at 1.56 mg/m^2/dose (3 subjects with Grade 3 diarrhea and 1 with peripheral sensory neuropathy). Therefore, the MTD at this schedule was 1.3 mg/m^2/dose. In a 35-day treatment cycle with 4 weekly doses of VELCADE monotherapy, the MTD was 1.6 mg/m^2/dose and DLT included hypotension, tachycardia, diarrhea, and syncope.

In phase 1 clinical studies, anti-tumor activity was reported in subjects with NHL, multiple myeloma, Waldenström's Macroglobulinemia, squamous cell carcinoma of the nasopharynx, bronchoalveolar carcinoma of the lung, renal cell carcinoma, and prostate cancer.

The safety and efficacy of VELCADE in subjects with multiple myeloma were investigated in two phase 2 clinical studies, studies M34100-024 (subjects with first relapse) (Jagannath et al, 2004) and M34100-025 (subjects with second or greater relapse and refractory to their last prior therapy) (Richardson et al, 2003). In M34100-025, 202 heavily pre-treated subjects with refractory multiple myeloma after at least 2 previous treatments received VELCADE, 1.3 mg/m^2 on Days 1, 4, 8, and 11 of a 21-day treatment cycle. The European Group for Blood and Marrow Transplant (EBMT) response criteria, as described by Blade (Blade et al., 1998) were utilized to determine disease response. CRs were observed in 4% of subjects, with an additional 6% of patients meeting all criteria for CR but having a positive immunofixation test. PR or better was observed in 27% of subjects, and the overall response rate (CR, PR and minor response [MR] combined) was 35%. Seventy percent of subjects experienced stable disease or better.

The phase 3 study (M34101-039) (Richardson et al, 2005), also referred to as the APEX study, was designed to determine whether VELCADE provided benefit (time to progression [TTP], response rate, and survival) to patients with relapsed or refractory MM relative to treatment with high-dose dexamethasone. The study was also designed to determine the safety and tolerability of VELCADE relative to high-dose dexamethasone, and whether treatment with VELCADE was associated with superior clinical benefit and quality of life relative to high-dose dexamethasone. A total of 669 patients were enrolled and 663 patients received study drug (VELCADE: 331; dexamethasone: 332). Patients randomized to VELCADE received 1.3 mg/m^2 I.V. push twice weekly on days 1, 4, 8, and 11 of a 3-week cycle for up to eight treatment cycles as induction therapy, followed by 1.3 mg/m^2 VELCADE weekly on days 1, 8, 15, and 22 of a 5-week cycle for three cycles as maintenance therapy. Patients randomized to dexamethasone received oral dexamethasone 40 mg once daily on days 1 to 4, 9 to 12, and 17 to 20 of a 5-week cycle for up to four treatment cycles as induction therapy, followed by dexamethasone 40 mg once daily on days 1 to 4 followed of a 4-week cycle for five cycles as maintenance therapy. The European Group for Blood and Marrow Transplant (EBMT) response criteria, as described by Blade (Blade et al., 1998) were utilized to determine disease response. There was a 78% increase in TTP for the VELCADE arm. Median TTP was 6.2 months for the VELCADE arm and 3.5 months for the dexamethasone arm (P<.0001). CR (complete response) + PR (partial response) was 38% with VELCADE vs. 18% with dexamethasone (P<.0001). CR was 6% with VELCADE vs. <1% with dexamethasone (P<.0001). The CR + nCR rate was13% with VELCADE vs. 2% with dexamethasone. In patients who had received only one prior line of treatment (VELCADE: 132; dexamethasone: 119), CR + PR was 45% with VELCADE vs. 26% with dexamethasone (P=.0035). With a median 8.3 months of follow-up, overall survival was significantly longer (P=.0013) for patients on the VELCADE arm vs. patients on the dexamethasone arm. The probability of survival at one year was 80% for the VELCADE arm vs. 66% for the dexamethasone arm, which represented a 41% decreased relative risk of death in the first year with VELCADE (P=.0005). In patients who had received only one prior line of treatment, the probability of survival at one year was 89% for the VELCADE arm vs. 72% for the dexamethasone arm, which represented a 61% decreased relative risk of death in the first year with VELCADE (P=.0098). Updated response rates and survival data were reported for M34101-039 (Richardson ASH, 2005). The updated CR (complete response) + PR (partial response) rate was 43% with VELCADE. The CR + nCR rate was 16% with VELCADE. With a median 22 months of follow-up, overall survival was significantly longer for patients on the VELCADE arm

vs. patients on the dexamethasone arm. The median overall survival was 29.8 months (95% CI: 23.2, not estimable) for the VELCADE arm vs 23.7 months (95% CI: 18.7, 29.1) for the dexamethasone arm (hazard ratio = 0.77, *P*= 0.0272). The probability of survival at one year was 80% for the VELCADE arm vs. vs 67% for the dexamethasone arm (*P*=0.0002).

The safety and efficacy of VELCADE in relapsed or refractory mantle cell lymphoma (MCL) were investigated in an international, phase 2, multicenter study M34103-053, also referred to as the PINNACLE study(Fisher et al, 2006). The single-arm study was designed to evaluate the response rates, duration of response (DOR), TTP, overall survival (OS), and safety of VELCADE treatment in patients with relapsed or refractory mantle cell lymphoma. For 141 evaluable patients, the response rate was 31% (8% CR/unconfirmed CR [Cru]). Median time to response was 40 days (range 31-204 days). The median number of cycles administered across all patients was 4; in responding patients, the median number of cycles was 8. The median DOR by algorithm was 9.2 months and 13.5 months in patients with CR/CRu. Median TTP for both groups was 6.2 months. With a median follow-up of 13.4 months, overall survival had not been reached. The most commonly reported adverse events (AEs) were fatigue, peripheral neuropathy, and gastrointestinal events. A time-to-event update to the PINNACLE study (Goy et al, 2007) was reported after a median follow-up of 26.4 months. TTP was 6.7 months for all patients, 12.4 months in all responders. The median DOR was 9.2 months in all responders and had not been reached in patients achieving CR/Cru. Overall survival was 23.5 months in all patients and 36 months in patients with CR/Cru. Survival at 12 months was 69% overall and 91% in responding patients.

The phase 3 study (MMY 3002) known as the VISTA study, evaluated the safety and efficacy of the combination of VELCADE, melphalan, and prednisone in previously untreated multiple myeloma patients who were not candidates for stem cell transplant (San Miguel, 2008). The study was designed to determine the benefit of adding VELCADE to MP (melphalan and prednisone) as assessed by TTP. Patients (682) were randomized to receive nine 6-week cycles of melphalan 9mg/m^2 and prednisone 60 mg/m^2 on Days 1 to 4, alone or in combination with VELCADE 1.3 mg/m^2 by IV bolus on Days 1, 4, 8, 11, 22, 25, 29, and 32 during Cycles 1 to 4, and on Days 1, 8, 22, and 29 during Cycles 5 to 9. Response was evaluated every 3 weeks using the EBMT criteria. At a preplanned interim analysis, the independent data monitoring committee recommended that the study be stopped since the prespecified statistical boundary end point of TTP had been crossed. Response rates were 30% with 4% CR. The rates of partial response or better were 71% in the VELCADE (VMP) group compared to 34% in the MP group (p = 0.001). With follow-up of 16.3 months, the TTP for the VMP group was 24 months compared to 16.6 months in the MP group (p = 0.000001) and was associated with a 52% reduced time to progression. The median DOR was 19.9 months in the VMP group and 13.1 months in the MP group. Overall survival had not been reached in either group. Hematologic toxicity was similar in both groups. The incidence of peripheral sensory neuropathy and gastrointestinal symptoms was higher in the VMP group. The incidence of herpes zoster was 3% in patients in the VMP group who received antiviral prophylaxis. Fifteen percent of patients in the VMP group discontinued therapy due to AEs compared to 14% in the MP group.

The VISTA study update after extended follow-up of 25.9 months, (San Miguel et al, 2008) confirmed a survival benefit for the VMP group. Overall survival was not reached in either group: VMP group (75) deaths, 3 year OS 72%; MP group (111) deaths, 3 year OS 59% ($p = 0.0032$). Patients on VMP were less likely to start second-line therapy (VMP 38% vs MP 57% at the time of data cut-off) with a longer time to next therapy (TNT) and treatment free interval (TFI). Of the MP patients who received subsequent therapy, 43% went on to receive VELCADE.

Based on investigator-reported best responses to subsequent therapies, patients relapsing after therapy with a novel agent were not intrinsically more resistant than after receiving a traditional agent.

In the VISTA study, VMP was associated with prolonged TTP, TNT, TFI, and OS. Patients were successfully treated with subsequent IMiD-based therapy and retreated with VELCADE. After 36.7 months follow-up, OS continued to be superior for VMP. The OS for VMP had not yet been reached compared to MP (43.1 months) (Mateos et al, 2009). In an updated analysis of overall survival based on 387 deaths (median follow-up 60.1 months), the median overall survival for VMP was 56.4 months and the MP was 43.1 months, with a hazard ration of 0.695 (95% CI: 0.57, 0.85).

VELCADE in mantle cell and indolent lymphomas

VELCADE was investigated in two phase II trials in relapsed/refractory NHL. O'Connor et al (2005) found 58% ORR with bortezomib administered at 1.5 mg/m^2 on Days 1, 4, 8, and 11 of a 21-day treatment cycle. Responses were seen in mantle cell, follicular and marginal zone lymphomas. Goy et al (2005) tested the same dose of bortezomib in a larger cohort of 60 patients and found 41% ORR in mantle cell lymphomas and 19% in other NHL subtypes. Dose of 1.3 mg/m^2 on Days 1, 4, 8, and 11 of a 21-day treatment cycle was examined in additional phase II trials, again showing response in mantle cell and indolent lymphomas (Strauss et al, 2006). A small phase II trial just in mantle cell lymphoma, using bortezomib at the dose of 1.3 mg/m^2 on Days 1, 4, 8, and 11 of a 21-day treatment cycle, showed a 46% ORR and median response duration of 10 months (Belch et al, 2007). A large phase II study (PINNACLE trial) in 155 patients with relapsed/refractory mantle cell lymphoma, demonstrated 31% ORR with 8% CR/Cru, with median duration of response of 9.3 months (Fisher et al, 2006). Based on this data, VELCADE was approved by the FDA in December 2006 as a single agent in the treatment of relapsed/refractory mantle cell lymphoma.

Lymphoplasmacytic lymphoma (Waldernstrom's macroglobulinemia) had dedicated trials demonstrating efficacy of bortezomib. Phase II trial by Chen et al (2007) using standard dosing of bortezomib revealed a 26% ORR, all partial responses by combined IgM and bidimensional measurement criteria. Treon et al (2007), on the other side, demonstrated an 85% ORR, but also without CRs. Responses in SLL/CLL are less robust but present, as indicated by phase II trial in fludarabine-refractory CLL (Faderl et al, 2006).

1.2.6 **Potential Risks of VELCADE**

To date, more than 300,000 patients have been treated with VELCADE in both clinical trials investigating its use in hematological malignancies and solid tumors, and in patients who were treated with commercially available VELCADE.

Prescribing physicians and health care practitioners are referred to their locally approved product label for VELCADE regarding Indications and Usage, Contraindications, Warnings, and Precautions.

The known anticipated risks of VELCADE therapy are presented in Table 1-1 and Table 1-2. These risks are grouped according to the combined frequency observed in an integrated analysis of AEs in sponsored clinical studies of single-agent VELCADE dosed at 1.3 mg/m^2 twice weekly on a 21-day schedule, in patients with multiple myeloma and mantle cell lymphoma.

Precautions and Restrictions

It is not known what effects VELCADE has on human pregnancy or development of the embryo or fetus. Therefore, female patients participating in this study should avoid becoming pregnant, and male patients should avoid impregnating a female partner. Nonsterilized female patients of reproductive age and male patients should use effective methods of contraception through defined periods during and after study treatment as specified below.

Female patients must meet 1 of the following:

- Postmenopausal for at least 1 year before the screening visit, or
- Surgically sterile, or
- If they are of childbearing potential, agree to practice 2 effective methods of contraception from the time of signing the informed consent form through 30 days after the last dose of VELCADE, or agree to completely abstain from heterosexual intercourse.

It is strongly recommended that at least 1 of these 2 methods be highly effective (see examples below).

Highly effective methods	Other effective methods (barrier methods)
Intra-uterine devices (IUD)	Latex condom
Hormonal contraceptives (birth control pills/oral contraceptives, injectable contraceptives, contraceptive patches, or contraceptive implants)	Diaphragm with spermicide Cervical cap Sponge

If one of the highly effective methods cannot be used, using 2 effective methods at the same time is recommended.

Male patients, even if surgically sterilized (ie, status postvasectomy) must agree to 1 of the following:

- Practice effective barrier contraception during the entire study treatment period and through a minimum of 30 days after the last dose of study drug, or completely abstain from heterosexual intercourse.

Table 1-1 Known Anticipated Risks of VELCADE by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class Observed Incidence	Preferred Term
Blood and Lymphatic System Disorders	
Most common	Thrombocytopenia*, anaemia*
Very common	Neutropenia*
Common	Lymphopenia, pancytopenia*, leukopenia*, febrile neutropenia
Cardiac Disorders	
Common	Tachycardia, atrial fibrillation, palpitations, cardiac failure congestive*
Uncommon	Cardiogenic shock*, atrial flutter, cardiac tamponade*±, bradycardia, atrioventricular block complete, arrhythmia, cardiac arrest*, cardiac failure, arrhythmia, pericardial effusion, pericarditis, pericardial disease±, cardiopulmonary failure±
Ear and Labyrinth Disorders	
Uncommon	Deafness, hearing impaired
Eye Disorders	
Common	Blurred vision, conjunctivitis, conjunctival haemorrhage
Gastrointestinal Disorders	
Most common	Constipation, diarrhoea*, nausea, vomiting*
Very common	abdominal pain (excluding oral and throat)
Common	Dyspepsia, pharyngolaryngeal pain, gastroesophageal reflux, abdominal distension, gastritis, stomatitis, mouth ulceration, dysphagia, gastrointestinal haemorrhage*, lower gastrointestinal haemorrhage*± rectal haemorrhage
Uncommon	Eructation, gastrointestinal pain, tongue ulceration, retching, upper gastrointestinal haemorrhage*, haematemesis*, oral mucosal petechiae, ileus paralytic*, ileus, odynophagia, enteritis, colitis, oesophagitis, enterocolitis, diarrhoea haemorrhagic, acute pancreatitis*, intestinal obstruction
General Disorders and Administration Site Conditions	
Most common	Fatigue, pyrexia
Very common	Chills, oedema peripheral, asthenia
Common	Neuralgia, lethargy, malaise, chest pain, mucosal inflammation*
Uncommon	Injection site pain, injection site irritation, injection site phlebitis, general physical health deterioration*, catheter-related complication
Hepatobiliary Disorders	
Uncommon	Hyperbilirubinaemia, hepatitis*±
Immune System Disorders	
Uncommon	Drug hypersensitivity, angioedema

Table 1-1 Known Anticipated Risks of VELCADE by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class Observed Incidence	Preferred Term
Infections and Infestations	
Very common	Upper respiratory tract infection, nasopharyngitis, pneumonia*, Herpes zoster*
Common	Lower respiratory tract infection*, sinusitis, pharyngitis, oral candidiasis, urinary tract infection*, sepsis*, bactaeremia*, cellulitis*, Herpes simplex, bronchitis, gastroenteritis*, infection
Uncommon	Septic shock*, catheter-related infection*, skin infection*, Herpes zoster disseminated*, lung infection*, infusion site cellulitis, catheter site cellulitis, infusion site infection, urosepsis*, Aspergillosis*, tinea infection, Herpes zoster ophthalmic, Herpes simplex ophthalmic, meningoencephalitis herpetic±, varicella, empyema±, fungal oesophagitis±
Injury, Poisoning, and Procedural Complications	
Common	Fall
Uncommon	Subdural haematoma
Investigations	
Common	Weight decreased, alanine aminotransferase (ALT) increased, aspartate aminotransferase (AST) increased, blood alkaline phosphatase increased, liver function test abnormal, blood creatinine increased*
Uncommon	Gamma-glutamyltransferase (GGT) increased, oxygen saturation decreased*, blood albumin decreased, ejection fraction decreased*
Metabolism and Nutritional Disorders	
Very common	Decreased appetite, anorexia, dehydration*
Common	Hyperglycaemia, hypoglycaemia, hyponatraemia, hypokalaemia, hypercalcaemia*
Musculoskeletal and Connective Tissue Disorders	
Very common	Bone pain, myalgia, arthralgia, back pain
Common	Muscular weakness
Uncommon	Limb discomfort
Neoplasms, Benign, Malignant, and Unspecified (including cysts and polyps)	
Uncommon	Tumour lysis syndrome*
Nervous System Disorders	
Most common	Peripheral neuropathy (including all preferred terms under the MedDRA High-level term Peripheral neuropathy NEC)
Very common	Paresthesia, dizziness excluding vertigo, headache
Common	Polyneuropathy, syncope, dysesthesia, dysgeusia, postherpetic neuralgia

Table 1-1 Known Anticipated Risks of VELCADE by MedDRA System Organ Class, Observed Incidence, and Preferred Term

System Organ Class Observed Incidence	Preferred Term
Uncommon	Convulsion, loss of consciousness, ageusia, encephalopathy, paralysis*,autonomic neuropathy, reversible posterior leukoencephalopathy syndrome±, posterior reversible encephalopathy syndrome ϕ
Psychiatric Disorders	
Very common	Anxiety, insomnia
Common	Confusional state
Uncommon	Delirium
Renal and Urinary Disorders	
Common	Renal impairment*, renal failure*, haematuria
Uncommon	Micturition disorder
Respiratory, Thoracic, and Mediastinal Disorders	
Very common	Cough, dyspnoea
Common	Epistaxis, dyspnoea exertional, pleural effusion*, rhinorrhea, hypoxia*, pulmonary oedema*
Uncommon	Hemoptysis*, acute respiratory distress syndrome*, respiratory failure*, pneumonitis*, lung infiltration, pulmonary alveolar haemorrhage*, interstitial lung disease*, pulmonary hypertension*, pleurisy, pleuritic pain
Skin and Subcutaneous Tissue Disorders	
Very common	Rash
Common	Rash pruritic, rash erythematous, urticaria, petechiae
Uncommon	Cutaneous vasculitis, leukocytoclastic vasculitis±
Vascular Disorders	
Common	Hypotension*, orthostatic hypotension
Uncommon	Cerebral haemorrhage*

Source: VELCADE® Investigator's Brochure Edition 15.

Most common = ≥ 30%, Very common = 10% to 29%, Common = 1% to 9%, Uncommon = < 1%.

* Fatal outcomes have been reported.

± Indicates a Preferred term not listed in the source table, however the event is deemed medically important and so is included.

ϕ Effective MedDRA update to version 14.0, the term 'reversible posterior leukoencephalopathy syndrome' updated to 'posterior reversible encephalopathy syndrome (PRES)'.

Table 1-2 Reports of Adverse Reactions From Postmarketing Experience

System Organ Class Preferred Term	Observed Incidence[a]
Blood and lymphatic system disorders	
Disseminated intravascular coagulation	Rare
Cardiac Disorders	
Atrioventricular block complete	Rare
Cardiac tamponade	Rare
Ear and labyrinth disorders	
Deafness bilateral	Rare
Eye Disorders	
Ophthalmic herpes	Rare
Optic neuropathy	Rare
Blindness	Rare
Gastrointestinal Disorders	
Acute pancreatitis	Rare
Ischemic colitis	Rare
Hepatobiliary disorders	
Hepatitis	Uncommon
Liver failure	Unknown
Infections and infestations	
Herpes meningoencephalitis	Rare
Septic shock	Rare
Progressive multifocal leukoencephalopathy	Very rare
Immune System Disorders	
Angioedema	Rare
Nervous System Disorders	
Autonomic neuropathy	Rare
Dysautonomia	Unknown
Encephalopathy	Rare
Respiratory, thoracic and mediastinal disorders:	
Acute diffuse infiltrative pulmonary disease[b]	Rare
Acute respiratory distress syndrome (ARDS)	Rare
Interstitial pneumonia	Rare
Lung infiltration	Rare
Pneumonitis	Rare
Pulmonary hypertension	Rare

Table 1-2 Reports of Adverse Reactions From Postmarketing Experience

System Organ Class Preferred Term	Observed Incidence[a]
Skin and subcutaneous system disorders	
Acute febrile neutrophilic dermatosis	Unknown
Toxic epidermal necrolysis	Unknown

Source: VELCADE® Investigator's Brochure Edition 15 Addendum 1.

a Incidence is assigned using the following convention: very common (≥ 1/10); common (≥ 1/100 and < 1/10); uncommon (≥ 1/1000 and < 1/100); rare (≥ 1/10,000 and < 1/1000); very rare (< 1/10,000, including isolated reports).

b Acute diffuse infiltrative pulmonary disease is a MedDRA Lower Level Term which corresponds to a Preferred Term of Interstitial lung disease.

Other medical events of interest that are considered not causally related to VELCADE include hepatic failure and QT prolongation. Fatal outcomes have been reported.

Women of childbearing potential should avoid becoming pregnant while being treated with VELCADE. Genotoxicity testing has shown that bortezomib is negative in the in vitro Ames assay and in the in vivo micronucleus assay, but it is a clastogen in the in vitro chromosomal aberration assay.

Additional details on the potential risks of VELCADE may be found in the Investigator's Brochure.

1.3 Combination drug information

1.3.1 Scientific Background

Cladribine

Leustatin (cladribine) Injection (also commonly known as 2-chloro-2'-deoxy-B-D-adenosine) is a synthetic antineoplastic agent for continuous intravenous infusion. It is a clear, colorless, sterile, preservative-free, isotonic solution. Leustatin Injection is available in single-use vials containing 10 mg (1 mg/mL) of cladribine, a chlorinated purine nucleoside analog. Each milliliter of Leustatin Injection contains 1 mg of the active ingredient and 9 mg (0.15 mEq) of sodium chloride as an inactive ingredient. The solution has a pH range of 5.5 to 8.0. Phosphoric acid and/or diabetic sodium phosphate may have been added to adjust the pH to 6.3±0.3.

The chemical name for cladribine is 2-chloro-6-amino-9-(2-deoxy-B-D-erythropento-furanosyl) purine.

The selective toxicity of 2-chloro-2'-deoxy-b-D-adenosine towards certain normal and malignant lymphocyte and monocyte populations is based on the relative activities of deoxycytidine kinase and deoxynucleotidase. Cladribine passively crosses the cell membrane. In cells with a high ratio of deoxycytidine kinase to deoxynucleotidase, it is phosphorylated by deoxycytidine kinase to 2-

chloro-2'-deoxy-B-D-adenosine monophosphate (2-CdAMP). Since 2-chloro-2'deoxy-β-D-adenosine is resistant to deamination by adenosine deaminase and there is little deoxynucleotide deaminase in lymphocytes and monocytes, 2-CdAMP accumulates intracellularly and is subsequently converted into the active triphosphate deoxynucleotide, 2-chloro-2'deoxy-β-D-adenosine triphosphate (2-CdATP). It is postulated that cells with high deoxycytidine kinase and low deoxynucleotidase activities will be selectively killed by 2-chloro-2'deoxy-β-D-adenosine as toxic deoxynucleotides accumulates intracellularly.

Cells containing high concentrations of deoxynucleotides are unable to properly repair single-strand DNA breaks. The broken ends of DNA activate the enzyme poly (ADP-ribose) polymerase resulting in NAD and ATP depletion and disruption of cellular metabolism. There is evidence, also, that 2-CdATP is incorporated into the DNA of dividing cells, resulting in impairment of DNA synthesis. Thus, 2-chloro-2'deoxy-β-D-adenosine can be distinguished from other chemotherapeutic agents affecting purine metabolism in that it is cytotoxic to both actively dividing and quiescent lymphocytes and monocytes, inhibiting both DNA synthesis and repair.

Rituximab

Rituximab is a mouse/human chimeric monoclonal antibody consisting of human IgG1 heavy and kappa light chain constant regions with murine variable regions from the murine IgG1 kappa anti-human CD20 monoclonal antibody rituximab. The rituximab antibody is produced by a Chinese hamster ovary transfectoma.

Rituximab binds specifically to the antigen CD20 (human B-lymphocyte-restricted differentiation antigen, Bp35), a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD located on pre-B and mature B lymphocytes. The antigen is expressed on >90% of B-cell non-Hodgkin's lymphomas (NHL), but the antigen is not found on hematopoietic stem cells, pro-B-cells, normal plasma cells or other normal tissues. CD20 regulates an early step(s) in the activation process for cell cycle initiation and differentiation, and possibly functions as a calcium ion channel. CD20 is not shed from the cell surface and does not internalize upon antibody binding. Free CD20 antigen is not found in the circulation.

B-cells are believed to play a role in the pathogenesis of rheumatoid arthritis (RA) and associated chronic synovitis. In this setting, B-cells may be acting at multiple sites in the autoimmune/inflammatory process, including through production of rheumatoid factor (RF) and other autoantibodies, antigen presentations, T-cell activation, and/or pro-inflammatory cytokine production.

Mechanism of Action: The Fab domain of rituximab binds to the CD20 antigen on B lymphocytes, and the Fc domain recruits immune effector functions to mediate B-cell lysis *in vitro*. Possible mechanisms of cell lysis include complement-dependent cytotoxicity (CDC) and antibody-dependent cell mediated cytotoxicity (ADCC). The antibody has been shown to induce apoptosis in the DHL-4 human B-cell lymphoma line.

Normal Tissue Cross-reactivity: Rituximab binding was observed on lymphoid cells in the thymus, the white pulp of the spleen, and a majority of B lymphocytes in peripheral blood and lymph nodes. Little or no binding was observed in the non-lymphoid tissues examined.

1.3.2 Clinical Pharmacokinetics and Pharmacodynamics

Cladribine

In a clinical investigation, 17 patients with Hairy Cell Leukemia and normal renal function were treated for 7 days with the recommended treatment regimen of Leustatin Injection (0.09 mg/kg/day) by continuous intravenous infusion. The mean steady-state serum concentration was estimated to be 5.7 ng/mL with an estimated systemic clearance of 663.5 mL/h/kg when Leustatin was given by continuous infusion over 7 days. In Hairy Cell Leukemia patients, there does not appear to be a relationship between serum concentrations and ultimate clinical outcome.

In another study, 8 patients with hematological malignancies received a two (2) hour infusion of Leustatin Injection (0.12 mg/kg). The mean end-of-infusion plasma Leustatin concentration was 48±19 ng/mL. For 5 of these patients, the disappearance of Leustatin could be described by either a biphasic or triphasic decline. For these patients with normal renal function, the mean terminal half-life was 5.4 hours. Mean values for clearance and steady-state volume of distribution were 978±422 mL/h/kg and 4.5±2.8 L/kg, respectively.

Cladribine plasma concentration after intravenous administration declines multi-exponentially with an average half-life of 6.7+2.5 hours. In general, the apparent volume of distribution of cladribine is approximately 9 L/kg, indicating an extensive distribution in body tissues.

Cladribine penetrates into cerebrospinal fluid. One report indicates that concentrations are approximately 25% of those in plasma.

Leustatin is bound approximately 20% to plasma proteins.

Except for some understanding of the mechanism of cellular toxicity, no other information is available on the metabolism of Leustatin in humans. An average of 18% of the administered dose has been reported to be excreted in urine of patients with solid tumors during a 5-day continuous intravenous infusion of 3.5-8.1 mg/m2/day of Leustatin. The effect of renal and hepatic impairment on the elimination of cladribine has not been investigated in humans.

Rituximab

Pharmacodynamics: Administration of Rituxan resulted in a rapid and sustained depletion of circulating and tissue-based B-cells. Among 166 patients in Study 1, circulating CD19-positive B-cells were depleted within the first three weeks with sustained depletion for up to 6 to 9 months post-treatment in 83% of patients. B-cell recovery began at approximately 6 months and median B-cell levels returned to normal by 12 months following completion of treatment.

There were sustained and statistically significant reductions in both IgM and IgG serum levels observed from 5 through 11 months following rituximab administration; 14% of patients had IgM and/or IgG serum levels below the normal range.

In RA patients, treatment with Rituxan induced depletion of peripheral B lymphocytes, with all patients demonstrating near complete depletion within 2 weeks after receiving the first dose of Rituxan. The majority of patients showed peripheral B-cell depletion for at least 6 months, followed by subsequent gradual recovery after that time point. A small proportion of patients (4%) had prolonged peripheral B-cell depletion lasting more than 3 years after a single course of treatment.

In RA studies, total serum immunoglobulin levels, IgM, IgG, and IgA were reduced at 6 months with the greatest change observed in IgM. However, mean immunoglobulin levels remained within normal levels over the 24-week period. Small proportions of patients experienced decreases in IgM (7%), IgG (2%), and IgA (1%) levels below the lower limit of normal. The clinical consequences of decreases in immunoglobulin levels in RA patients treated with Rituxan are unclear.

Treatment with rituximab in patients with RA was associated with reduction of certain biologic markers of inflammation such as interleukin-6 (IL-6), C-reactive protein (CRP), serum amyloid protein (SAA), s100 A8/S100 A9 heterodimer complex (S100 A8/9), anti-citrullinated peptide (anti-CCP) and RF.

Pharmacokinetics: Pharmacokinetics were characterized in 203 NHL patients receiving 375 mg/m^2 rituximab weekly by IV infusion for 4 doses. The mean C_{max} increased with each successive infusion and was 486 mcg/mL (range, 78-997 mcg/mL) following the fourth infusion. Peak and trough serum levels of rituximab were inversely correlated with pretreatment circulating CD19-positive B-cells and tumor burden. Rituximab was detectable in the serum of patients 3 to 6 months after completion of treatment.

The pharmacokinetic profile of rituximab when administered as 6 infusions of 375 mg/m^2 in combination with 6 cycles of CHOP chemotherapy was similar to that seen with rituximab alone.

Based on a population pharmacokinetic analysis of data from 298 NHL patients who received rituximab once weekly or once every three weeks, the estimated median terminal elimination half-life was 22 days (range, 6.1 to 52 days). Patients with higher CD19-positive cell counts or larger measurable tumor lesions at pretreatment had a higher clearance. However dose adjustment for pretreatment CD19 count or size of tumor lesion is not necessary. Age and gender had no effect on the pharmacokinetics of rituximab.

Following administration of 2 doses of rituximab in patients with rheumatoid arthritis, the mean C_{max} values were 183 mcg/mL (CV=24%) for the 2 X 500 mg dose and 370 mcg/mL (CV=25%) for the 2 X 1000 mg dose, respectively. Following 2 X 1000 mg rituximab dose, mean volume of distribution at steady state was 4.3 L (CV=28%). Mean systemic clearance of rituximab was 0.01 L/h (CV=38%), and mean terminal elimination half-life after the second dose was 19 days (CV=32%).

Female patients with RA (n=86) had a 37% lower clearance of rituximab than male patients with RA (n=25). The gender difference in rituximab clearance does not necessitate any dose adjustment because safety and efficacy of rituximab do not appear to be influenced by gender.

The pharmacokinetics of rituximab have not been studied in children and adolescents. No formal studies were conducted to examine the effects of either renal or hepatic impairment on the pharmacokinetics of rituximab.

1.3.3 Clinical Experience

Cladribine

Cladribine is a purine nucleoside analog effective in indolent and mantle cell lymphomas. Experience at the Mayo Clinic and the University of Arizona Cancer Center indicate cladribine

to be extremely effective in mantle cell lymphomas with tolerable toxicity, complete response rates of 30-50%, and durability of responses at 19-24 months in newly diagnosed patients, as compared to 12-15 months with CHOP (Inwards et al, 2008; Rummel et al., 1999). In indolent lymphomas, cladribine has resulted in CR rates of up to 20% in relapsed and up to 32% in untreated setting (Kay et al., 1992; Fridrik et al., 1998). The FDA approval for cladribine is based on its efficacy in treatment of hairy cell leukemia, an indolent lymphoid malignancy.

Cladribine in hairy cell leukemia

Two single-center open label studies of Leustatin (cladribine) have been conducted in patients with Hairy Cell Leukemia with evidence of active disease requiring therapy. In the study conducted at the Scripps Clinic and Research Foundation (Study A, Saven et al, 1998), 89 patients were treated with a single course of Leustatin Injection given by continuous infusion for 7 days at a dose of 0.09 mg/kg/day. In the study conducted at the M.D. Anderson Cancer Center (Study B, Estey et al., 1992), 35 patients were treated with a 7-day continuous intravenous infusion of Leustatin Injection at a comparable dose of 3.6 mg/m^2/day. A complete response (CR) required clearing of the peripheral blood and bone marrow of hairy cells and recovery of the hemoglobin to 12 g/dL, platelet count to 100 x 10^9/L, a absolute neutrophil count to 1500 x 10^6/L. A good partial response (GPR) required the same hematologic parameters as a complete response, and that fewer than 5% hairy cells remain in the bone marrow. A partial response (PR) required that hairy cells in the bone marrow be decreased by at least 50% from baseline and the same response for hematologic parameters as for complete response. A pathologic relapse was defined as an increase in bone marrow hairy cells to 35% of pretreatment levels. A clinical relapse was defined as the recurrence of cytopenias, specifically, decreases in hemoglobin $\geq$2 g/dL, ANC$\geq$25% or platelet counts $\geq$50,000. Patients who met the criteria fro a complete response but subsequently were found to have evidence of bone marrow hairy cells (<25% of pretreatment levels) were reclassified as partial responses and were not considered to be complete responses with relapse.

Among patients evaluable for efficacy (N=106), using the hematologic and bone marrow response criteria described above, the complete response rates in patients treated with Leustatin Injection were 65% and 68% for Study A and Study B, respectively, yielding a combined complete response rate of 66%. Overall response rates (i.e., Complete plus Good Partial plus Partial Responses) were 89% and 86% in Study A and Study B, respectively, for a combined overall response rate of 88% in evaluable patients treated with Leustatin Injection.

Using an intent-to-treat analysis (N=123) and further requiring no evidence of splenomegaly as a criterion for CR (i.e., no palpable spleen on physical examination and $\leq$13 cm on CT scan), the complete response rates for Study A and Study B were 54% and 53%, respectively, giving a combined CR rate of 54%. The overall response rates (CR + GPR + PR) were 90% and 85%, for Studies A and B, respectively, yielding a combined overall response rate of 89%. In these studies, 60% of the patients had not received prior chemotherapy for Hairy Cell Leukemia or had undergone splenectomy as the prior treatment and were receiving Leustatin as a first-line treatment. The remaining 40% of the patients received Leustatin as a second-line treatment, having been treated with other agents, including α-interferon and/or deoxycofomycin. The overall response rate for patients without prior chemotherapy was 92%, compared with splenectomy or deoxycofomycin and in patients refractory to α-interferon.

After a reversible decline, normalization of peripheral blood counts (Hemoglobin >12.0 g/dL, Platelets >100 x 10^9/L, Absolute Neutrophil Count (ANC) >1500 x 10^6/L) was achieved by 92% of evaluable patients. The median time to normalization of peripheral counts was 9 weeks from the start of treatment (Range: 2 to 72). The median time to normalization of Platelet Count was 2 weeks, the median time to normalization of the ANC was 5 weeks and the median time to normalization of Hemoglobin was 8 weeks. With normalization of Platelet Count and Hemoglobin, requirements for platelet and RBC transfusions were abolished after Months 1 and 2, respectively, in those patients with complete response. Platelet recovery may be delayed in a minority of patients with severe baseline thrombocytopenia. Corresponding to normalization of ANC, a trend toward a reduced incidence of infection was seen after the third month, when compared to the months immediately preceding Leustatin therapy.

For patients achieving a complete response, the median time to response (i.e., absence of hairy cells in bone marrow and peripheral blood together with normalization of peripheral blood parameters), measured from treatment start, was approximately 4 months. Since bone marrow aspiration and biopsy were frequently not performed at the time of peripheral blood normalization, the median time to complete response may actually be shorter than that which was recorded. At the time of data cut-off, the median duration of complete response was greater than 8 months and ranged to 25+ months. Among 93 responding patients, seven had shown evidence of disease progression at the time of the data cut-off. In four of these patients, disease was limited to the bone marrow without peripheral blood abnormalities (pathologic progression), while in three patients there were also peripheral blood abnormalities (clinical progression). Seven patients who did not respond to a first course of Leustatin received a second course of therapy. In the five patients who had adequate follow-up, additional courses did not appear to improve their overall response.

Cladribine in mantle cell lymphoma

Prior to realization that mantle cell lymphoma has unique characteristics separating it from indolent lymphomas, it was included in the same trials without differentiating survival outcomes, although the response rates were similar. Rummel et al (1999) treated 12 patients with mantle cell lymphoma (7 untreated, 5 relapsed), with cladribine at 5 mg/m2 IV over 2 hours days 1-5 every 4-5 weeks for 6 cycles. CR rate was 25% and PR was 33%, with remission duration of 19 months and 4-year OS of 58%. Grade 3-4 neutropenia was observed in 17% and grade 3-4 thrombocytopenia in only 2%. Inwards et al at the Mayo Clinic conducted a phase II trial using the same administration schedule in 24 previously treated patients, with CR/CRu rate of 21% and PR rate of 25%, with median PFS of 5.4 months and median OS of 1.9 years. In untreated patients, 42% CR and 39% PR rates were seen, with median PFS of 13.6 months and median OS of 4.7 years. When cladribine was combined with rituximab in a follow-up study of 29 patients with median age of 70, CR increased to 52%, PR was 14%, median PFS was 12.1 months, and median OS was not reached. Of 15 patients achieving CR, only 3 patients relapsed with the median follow-up of 21.5 months. Patients received a median of 4 cycles. Combination of cladribine and rituximab resulted in 7% grade 3 and 24% grade 4 neutropenia, as well as 14% grade 3 and 3% grade 4 thrombocytopenia. One patient died during treatment from a cerebrovascular incident following a pneumonia. (Inwards et al, 2008). Similar if not more impressive responses were seen

at the University of Arizona Cancer Center and Oregon Health and Science University (personal communication, Drs. Miller and Epner).

Cladribine in indolent lymphoma

Initial study by Kay et al (1992) in 40 patients, 29 refractory to prior therapy, using 7-day infusion schedule showed 20% CR and 23% PR with a median duration of response being 5 months. Grade 3-4 neutropenia and thrombocytopenia were observed in 18% and 30%, respectively, with only one patient with platelet count below 20,000/μL. There was cumulative myelosuppression and increased risk of infection, with 13% bacteremia. Further studies in relapsed/refractory indolent NHL confirmed ORR of 36-65% and CR rates of 4-20% (reviewed by Zinzani, 2002).

In untreated indolent lymphomas, cladribine was even more effective, with ORR 64-100% and CR rates of 7-32% (reviewed by Zinzani, 2002). The largest was by Fridrik et al (1998) in 50 patients, 6 of whom had mantle cell lymphoma, using 0.12 mg/kg IV 2-hour infusion days 1-5 of a 28 day cycle, for 4 cycles. ORR was 88%, 28% CR rate, with 21-month OS of 91% and 21-month time to treatment failure of 51%. Only 10% grade 3-4 neutropenia and 0% grade 3-4 thrombocytopenia were seen, with only 7 infections during treatment and 6 infections during the observation period. All mantle cell lymphoma patients responded (3 CRs, 3 PRs).

Cladribine has been studied much less than fludarabine, another purine analog, and until recently there was no head-to-head comparison of the two. Recent phase III trial in untreated symptomatic CLL randomized 229 patients from Scandinavia and Australia to cladribine 5 mg/m2 IV over 2 hours days 1-5; fludarabine 25 mg/m2 IV bolus days 1-5; or chlorambucil 10 mg/m2 orally days 1-10; on a 28 day cycle. Median time to progression was 25 months for cladribine, 10 months for fludarabine, and 9 months for chlorambucil (p=0.0003). The trend for median OS favored cladribine (82 months vs. 68 months for fludarabine) but was not statistically significant. (Karlsson et al., ASH 2007).

Rituximab

Rituxan® (Rituximab) is indicated for the treatment of patients with relapsed or refractory, low-grade or follicular, CD20-positive, B-cell, non-Hodgkin's lymphoma.

Rituxan® (Rituximab) is indicated for the first-line treatment of follicular, CD20-positive, B-cell non-Hodgkin's lymphoma in combination with CVP chemotherapy.

Rituxan® (Rituximab) is indicated for the treatment of low-grade, CD20-positive, B-cell non-Hodgkin's lymphoma in patients with stable disease or who achieve a partial or complete response following first-line treatment with CVP chemotherapy.

Rituxan® (Rituximab) is indicated for the first-line treatment of diffuse large B-cell, CD20-positive, non-Hodgkin's lymphoma in combination with CHOP or other anthracycline-based chemotherapy regimens.

Relapsed or Refractory, Low-Grade or Follicular, CD20-Positive, B-Cell NHL

The safety and effectiveness of Rituxan in relapsed, refractory CD20+ NHL were demonstrated in 3 single-arm studies enrolling 296 patients.

Study 1

A multicenter, open-label, single-arm study was conducted in 166 patients with relapsed or refractory, low-grade or follicular B-cell NHL who received 375 mg/m^2 of Rituxan given as an intravenous infusion weekly for 4 doses (McLaughlin et al., 1998). Patients with tumor masses >10cm or with >5000 lymphocytes/μL in the peripheral blood were excluded from the study.

Results are summarized in Table #1. The median time to onset of response was 50 days. Disease-related signs and symptoms (including B-symptoms) resolved in 64% (25/39) of those patients with such symptoms at study entry.

Study 2

In a multicenter, single-arm study, 37 patients with relapsed or refractory, low-grade NHL received 375 mg/m^2 of Rituxan weekly for 8 doses (Maloney et al., 1997). Results are summarized in Table #1.

Study 3

In a multicenter, single-arm study, 60 patients received 375 mg/m^2 of Rituxan weekly for 4 doses. All patients had relapsed or refractory, low-grade or follicular B-cell NHL and had achieved an objective clinical response to Rituxan administered 3.8-35.6 months (median 14.5 months) prior to retreatment with Rituxan (Davis et al., 2000). Of these 60 patients, 5 received more than one additional course of Rituxan. Results are summarized in Table #1.

Bulky Disease

In pooled data from studies one and three, 39 patients with bulky (single lesion >10cm in diameter) and relapsed or refractory, low-grade NHL received Rituxan 375 mg/m^2 weekly for 4 doses (Davis et al., 1999). Results are summarized in Table 2.

Table 2

Summary of Rituxan Data by Schedule and Clinical Setting

	Study 1 Weekly x 4 N=166	Study 2 Weekly x 8 N=37	Study 1 and 3 Bulky disease Weekly x 4 N=39[a]	Study 3 Retreatment, Weekly x 4 N=60
Overall Response Rate	48%	57%	36%	38%
Complete Response Rate	6%	14%	3%	10%
Median Duration of Response [b,c,d] (months) [Range]	11.2 [1.9 to 42.1+]	13.4 [2.5 to 36.5+]	6.9 [2.8 to 25.0+]	15.0 [3.0 to 25.1+]

[a] Six of these patients are included in the first column. Thus, data from 296 intent to treat patients are provided in this table.

[b] Kaplan-Meier projected with observed range.

[c] "+" indicates an ongoing response.

[d] Duration of response: interval from the onset of response to disease progression.

Study 4

A total of 322 patients with previously untreated follicular NHL were randomized (1:1) to receive up to eight 3-week cycles of CVP chemotherapy alone (CVP) or in combination with Rituxan 375 mg/m^2 on Day 1 of each cycle (R-CVP) in an open-label, multicenter study (Marcus et al., 2005). The main outcome measure of the study was progression-free survival (PFS) defined as the time from randomization to the first of progression, relapse, or death.

Twenty-six percent of the study population was >60 years of age, 99% had Stage III or IV disease, and 50% had an International Prognostic Index (IPI) score ≥2. The results for PFS as determined by a blinded, independent assessment of progression are presented in Table 3. The point estimates may be influenced by the presence of informative censoring. The PFS results based on investigator assessment of progression were similar to those obtained by the independent review assessment.

Table 3

		Study Arm	
	R-CVP N=162		CVP N=160
Median PFS (years)[a]	2.4		1.4
Hazard ratio (95%CI)[b]		0.44 (0.29, 0.65)	

[a] p <0.0001, two-sided stratified log-rank test.

[b] Estimates of Cox regression stratified by center.

Non-Progressing Low-Grade. CD20-Positive, B-Cell NHL Following First-Line CVP Chemotherapy

Study 5

A total of 322 patients with previously untreated low-grade, B-cell NHL who did not progress after 6 or 8 cycles of CVP chemotherapy were enrolled in an open-label, multicenter, randomized trial. Patients were randomized (1:1) to receive Rituxan, 375 mg/m^2 intravenous infusion, once weekly for 4 doses every 6 months for up to 16 doses or no further therapeutic intervention (Hochster et al., ASH 2005). The main outcome measure of the study was progression-free survival defined as the time from randomization to progression, relapse, or death. Thirty-seven percent of the study population was > 60 years of age, 88% has Stage III or IV disease, and 63% had an IPI score ≥2.

There was a reduction in the risk of progression, relapse, or death (hazard ratio estimate in the range of 0.36 to 0.49) for patients randomized to Rituxan as compared to those who received no additional treatment.

Rituximab in mantle cell lymphoma

In mantle cell lymphoma, rituximab seems to enhance the effects of combination chemotherapy but has only moderate activity as a single agent. Single agent rituximab given at 375 mg/m2 weekly for 4 doses was examined in 34 untreated and 40 patients with relapsed MCL. For untreated patients, ORR was 38%, CR rate was 16%, while for previously treated patients ORR was 37% and CR rate was 14%. Time to progression was 7 months, with response duration of 12

months (Foran et al, 2000). In a prospective phase III trial, 122 patients with newly diagnosed MCL were randomized to CHOP or CHOP combined with rituximab. Addition of rituximab improved ORR from 75% to 94%, CR rate from 7% to 34%; and median time to treatment failure from 14 to 21 months. However, there was no difference in PFS or OS (Lenz et al., 2005). On the other hand, when rituximab was added to a combination of fludarabine, cyclophosphamide and mitoxantrone (FCM) in a randomized prospective trial of relapsed MCL, it resulted in improved OS (not reached vs. 11 months) (Forstpointner et al., 2004).

1.3.4 Potential Risks of Cladribine and Rituximab

Cladribine

Severe bone marrow suppression, including neutropenia, anemia and thrombocytopenia, has been commonly observed in patients treated with Leustatin, especially at high doses. At initiation of treatment, most patients in the clinical studies had hematological impairment as a manifestation of active Hairy Cell Leukemia. Following treatment with Leustatin, further hematologic impairment occurred before recovery of peripheral blood counts began. During the first two weeks after treatment initiation, mean Platelet Count, ANC, and Hemoglobin concentration declined and subsequently increased with normalization of mean counts by Day 12, Week 5 and Week 8, respectively. The myelosuppressive effects of Leustatin were most notable during the first month following treatment. Forty-four percent (44%) of patients received transfusions with RBCs and 14% received transfusions with platelets during Month 1. Careful hematologic monitoring, especially during the first 4 to 8 weeks after treatment with Leustatin Injection, is recommended.

Fever ($T \geq 100^{o}F$) was associated with the use of Leustatin in approximately two-thirds of patients (131/196) in the first month of therapy. Virtually all of their patients were treated empirically with parenteral antibiotics. Overall, 47% (93/196) of all patients had fever in the setting of neutropenia ($ANC \leq 1000$), including 62 patients (32%) with severe neutropenia (i.e., $ANC \leq 500$).

In a Phase I investigational study using Leustatin in high doses (4 to 9 times the recommended dose for Hairy Cell Leukemia) as part of a bone marrow transplant conditioning regimen, which also included high dose cyclophosphamide and total body irradiation, acute nephrotoxicity and delayed onset neurotoxicity were observed.

Thirty-one (31) poor-risk patients with drug-resistant acute leukemia in relapse (29 cases) or non-Hodgkin's Lymphoma (2 cases) received Leustatin for 7 to 14 days prior to bone marrow transplantation. During infusion, 8 patients experienced gastrointestinal symptoms. While the bone marrow was initially cleared of all hematopoietic elements, including tumor cells, leukemia eventually recurred in all treated patients. Within 7 to 13 days after starting treatment with Leustatin, 6 patients (19%) developed manifestations of renal dysfunction (e.g., acidosis, anuria, elevated serum creatinine, etc.) and 5 required dialysis. Several of these patients were also being treated with other medications having known nephrotoxic potential. Renal dysfunction was reversible in 2 of these patients. In the 4 patients whose renal function had not recovered at the time of death, autopsies were performed; in 2 of these, evidence of tubular damage was noted. Eleven (11) patients (35%) experienced delayed onset neurologic toxicity. In the majority, this was characterized by progressive irreversible motor weakness (paraparesis/quadriparesis) of the upper and/or lower extremities, first noted 35 to 84 days after starting high dose therapy with

Leustatin. Non-invasive testing (electromyography and nerve conduction studies) was consistent with demyelinating disease. Severe neurologic toxicity has also been noted with high doses of another drug in this class.

Axonal peripheral polyneuropathy was observed in a dose fir Hairy Cell Leukemia) in patients not receiving cyclophosphamide or total body irradiation.

Severe neurological toxicity has been reported rarely following treatment with standard cladribine dosing regimens.

In patients with Hairy Cell Leukemia treated with the recommended treatment regimen (0.09 mg/kg/day for 7 consecutive days), there have been no reports of nephrologic toxicities.

Of the 196 Hairy Cell Leukemia patients entered in the two trials, there were 8 deaths following treatment. Of these, 6 were of infectious etiology, including 3 pneumonias, and 2 occurred in the first month following Leustatin therapy. Of the 8 deaths, 6 occurred in previously treated patients who were refractory to α interferon.

Benzyl alcohol is a constituent of the recommended diluent for the 7-day infusion solution. Benzyl alcohol has been reported to be associated with a fatal "Gasping Syndrome" in premature infants.

Teratogenesis, Carcinogenesis, Impairment of Fertility, Pregnancy, and Nursing:

Leustatin Injection should not be given during pregnancy.

Cladribine is teratogenic in mice and rabbits and consequently has the potential to cause fetal harm when administered to a pregnant woman. A significant increase in fetal variations was observed in mice receiving 1.5 mg/kg/day (4.5 mg/m^2) and increased resorptions, reduced litter size and increased fetal malformations were observed when mice received 3.0 mg/kg/day (9 mg/m^2). Fetal death and malformations were observed in rabbits that received 3.0 mg/kg/day (33.0 mg/m^2). No fetal effects were seen in mice at 0.5 mg/kg/day (1.5 mg/m^2) or in rabbits at 1.0 mg/kg/day (11.0 mg/m^2).

Although there is no evidence of teratogenicity in humans due to Leustatin, other drugs which inhibit DNA synthesis (e.g., methotrexate and aminopterin) have been reported to be teratogenic in humans. Leustatin has been shown to be embryotoxic in mice when given at doses equivalent to the recommended dose.

There are no adequate and well controlled studies in pregnant women. If Leustatin is used during pregnancy, of if the patient becomes pregnant while taking this drug, the patient should be apprised of the potential hazard to the fetus. Women of childbearing potential should be advised to avoid becoming pregnant.

No animal carcinogenicity studies have been conducted with cladribine. However, its carcinogenic potential cannot be excluded based on demonstrated genotoxicity of cladribine.

Cladribine was not mutagenic *in vitro* (Ames and Chinese hamster ovary cell gene mutation tests) and did not induce unscheduled DNA synthesis in primary rat hepatocyte cultures. However, cladribine was clastogenic both *in vitro* (chromosome aberrations in Chinese hamster ovary cells) and *in vivo* (mouse bone marrow micronucleus test).

When administered intravenously to Cynomolgus monkeys, cladribine has been shown to cause suppression of rapidly generating cells, including testicular cells. The effect on human fertility is unknown.

It is not known whether this drug is excreted in human milk. Because of the potential for serious adverse reactions in nursing infants, patients should not be nursing while receiving cladribine.

Adverse reactions:

Safety data are based on 196 patients with Hairy cell Leukemia: the original cohort of 124 patients plus an additional 72 patients enrolled at the same two centers after the original enrollment cutoff. In month 1 of Hairy cell Leukemia clinical trials, severe neutropenia was noted in 70% of patients, fever in 69%, and infection was documented in 28%. Other adverse experiences reported frequently during the first 14 days after initiating treatment included: fatigue (45%), nausea (28%), rash (27%), headache (22%), and injections site reactions (19%). Most non-hematologic adverse experiences were mild to moderate in severity.

Myelosuppression was frequently observed during the first month after starting treatment. Neutropenia (ANC $< 500 \times 10^6$/L) was noted in 70% of patients, compared with 26% in whom it was present initially. Severe anemia (hemoglobin < 8.5 g/dL) developed in 37% of patients, compared with 10% initially. Thrombocytopenia (platelets $< 20 \times 10^9$/L) developed in 12% of patients, compared to 4% in whom it was noted initially.

During the first month, 54 of 196 patients (28%) exhibited documented evidence of infection. Serious infections (e.g., septicemia, pneumonia) were reported in 6% of all patients; the remaining infections were mild or moderate. Several deaths were attributable to infection and/or complications related to the underlying disease. During the second month, the overall rate of documented infection was 6%; these infections were mild to moderate and no severe systemic infections were seen. After the third month, the monthly incidence of infection was either less than or equal to that of the months immediately preceding Leustatin therapy.

During the first month, 11% of patients experienced severe fever (i.e., ≥ 104ºF). Documented infections were noted in fewer than one-third of febrile episodes. Of the 196 patients studied, 19 were noted to have a documented infection in the month prior to treatment. In the month following treatment, there were 54 episodes of documented infection: 23 (42%) were bacterial, 11 (20%) were viral and 11 (20%) were fungal. Seven of 8 documented episodes of herpes zoster occurred during the month following treatment. Virtually all of these patients were treated empirically with antibiotics.

Analysis of lymphocyte subsets indicates that treatment with cladribine is associated with prolonged depression of the CD4 counts. Prior to treatment, the mean CD4 count was 766/µl. The mean CD4 count nadir, which occurred 4 to 6 months following treatment, was 272/µl. Fifteen months after treatment, mean CD4 counts remained below 500/µl. CD8 counts behaved similarly, though increasing counts were observed after 9 months. The clinical significance of the prolonged CD4 lymphopenia is unclear.

Another event of unknown clinical significance includes the observation of prolonged bone marrow hypocellularity. Bone marrow cellularity of < 35% was noted after 4 months in 42 of 124

patients (34%) treated in the two pivotal trials. This hypocellularity was noted as late as day 1010. It is not known whether the hypocellularity is the result of disease related marrow fibrosis or if it is the result of cladribine toxicity. There was no apparent clinical effect on the peripheral blood counts.

The vast majority of rashes were mild and occurred in patients who were receiving or had recently been treated with other medications (e.g., allopurinol or antibiotics) known to cause rash.

Most episodes of nausea were mild, not accompanied by vomiting, and did not require treatment with antiemetics. In patients requiring antiemetics, nausea was easily controlled, most frequently with chlorpromazine.

Adverse reactions reported during the first 2 weeks following treatment initiation (regardless of relationship to drug) by >5% of patients included:

Body as a whole: fever (69%), fatigue (45%), chills (9%), asthenia (9%), diaphoresis (9%), malaise (7%), trunk pain (6%)

Gastrointestinal: nausea (28%), decreased appetite (17%), vomiting (13%), diarrhea (10%), constipation (9%), abdominal pain (6%)

Hematologic/Lymphatic: purpura (10%), petechiae (8%), epistaxis (5%)

Nervous System: headache (22%), dizziness (9%), insomnia (7%)

Cardiovascular System: edema (6%), tachycardia (6%)

Respiratory System: abnormal breath sounds (11%), cough (10%), abnormal chest sounds (9%), shortness of breath (7%)

Skin/Subcutaneous Tissue: rash (27%), injections site reaction (19%), pruritus (6%), pain (6%), erythema (6%)

Musculoskeletal System: myalgia (7%), arthralgia (5%)

Adverse reactions related to intravenous administration included: injection site reactions (9%) (i.e., redness, swelling, pain), thrombosis (2%), phlebitis (2%), and a broken catheter (1%). These appear to be related to the infusion procedure and/or indwelling catheter, rather than the medication or the vehicle. From Day 15 to the last follow-up visit, the only events reported by >5% of patients were: fatigue (11%), rash (10%), headache (7%), cough (7%), and malaise (5%).

The following additional adverse events shave been reported since the drug became commercially available. These adverse events have been reported primarily in patients who received multiple course of Leustatin Injection:

Hematologic: bone marrow suppression with prolonged pancytopenia, including some reports of aplastic anemia; hemolytic anemia, which was reported in patients with lymphoid malignancies, occurring within the first few weeks following treatment. Rare cases of myelodysplastic syndrome have been reported.

Hepatic: reversible, generally mild increases in bilirubin and transaminases.

Nervous System: Neurological toxicity; however, severe neurotoxicity has been reported rarely

following treatment with standard cladribine dosing regimens.

Respiratory System: pulmonary interstitial infiltrates; in most cases, an infectious etiology was identified.

Skin/Subcutaneous: urticaria, hypereosinophilia. In isolated cases Stevens-Johnson and toxic epidermal necrolysis have been reported in patient who were receiving or had recently been treated with other medications (e.g., allopurinol or antibiotics) known to cause these syndromes.

Opportunistic infections have occurred in the acute phase of treatment due to the immunosuppression mediate by Leustatin Injection.

Rituximab

Human Toxicology: Single doses of up to 500 mg/m2 and weekly x 4 doses of 375 mg/m2 have been administered without dose limiting toxicity. Adverse events are most common during the initial antibody infusion and usually consist of Grade I or 2 fever (73%), asthenia (16%) chills (38%) nausea (19%), vomiting (11%), rash (14%) and tumor site pain (3%). Grade 1 or 2 hypotension (8%) may be treated with IV fluids. Hematologic toxicity is usually mild and reversible. Transient decreases in the WBC or platelet count have been observed - especially in patients with high levels of circulating tumor cells or bone marrow involvement. Two patients have had late-onset Grade 4 neutropenia at four and ten months that was attributed to an unknown cause, was transient and resolved. Infections (Grade 1 and 2) have not been related to dose level. Symptoms are generally associated with the initial antibody infusions and diminish in frequency with each successive infusion. A report in the literature described an increase in fatal infection in HIV-related lymphoma patients when rituximab was used in combination with CHOP chemotherapy as compared to CHOP alone. In patients with Waldenstrom's macroglobulinemia, following initiation of rituximab therapy, transient increases in serum IgM levels have been observed which may result in hyperviscosity syndrome requiring plasmapheresis.

Severe Infusion and Hypersensitivity Reactions: Rituximab has caused severe infusion reactions. In some cases, these reactions were fatal. An infusion-related symptom complex consisting of fever and chills/rigors has occurred in the majority of patients during the first rituximab infusion. Signs and symptoms of severe infusion reactions may include urticaria, hypotension, angioedema, hypoxia, or bronchospasm. The most severe manifestations and sequelae include pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, cardiogenic shock, and anaphylactic and anaphylactoid events. These reactions generally occurred within 30 minutes to 2 hours of beginning the first infusion, and resolved with slowing or interruption of the rituximab infusion and with supportive care (including, but not limited to IV saline, diphenhydramine, and acetaminophen).

Tumor Lysis Syndrome: Rituximab rapidly decreases benign and malignant CD20 positive cells. Tumor lysis syndrome has been reported to occur within 12 to 24 hours after the first rituximab infusion in patients with high numbers of circulating malignant lymphocytes. Patients with high tumor burden (bulky lesions) may also be at risk. Patients at risk for developing tumor lysis syndrome should be followed closely and appropriate laboratory monitoring performed.

Hepatitis B Reactivation with Related Fulminant Hepatitis and Other Viral Infections: Hepatitis B virus (HBV) reactivation with fulminant hepatitis, hepatic failure, and death has been reported in some patients with hematologic malignancies treated with rituximab. The majority of patients received rituximab in combination with chemotherapy. The median time to the diagnosis of hepatitis was approximately 4 months after the initiation of rituximab and approximately one month after the last dose. Persons at high risk of HBV infection should be screened before initiation of rituximab. Carriers of hepatitis B should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis during and for up to several months following rituximab therapy. In patients who develop viral hepatitis, rituximab and any concomitant chemotherapy should be discontinued and appropriate treatment including antiviral therapy initiated. There are insufficient data regarding the safety of resuming rituximab therapy in patients who develop hepatitis subsequent to HBV reactivation.

The following additional serious viral infections, either new, reactivated or exacerbated, have been identified in clinical studies or postmarketing reports. The majority of patients received rituximab in combination with chemotherapy or as part of a hematopoietic stem cell transplant. These viral infections included JC virus (progressive multifocal leukoencephalopathy [PML]), cytomegalovirus, herpes simplex virus, parvovirus B19, varicella zoster virus, West Nile virus, and hepatitis C. In some cases, the viral infections occurred up to one year following discontinuation of rituximab and have resulted in death.

Severe Mucocutaneous Reactions: Mucocutaneous reactions, some with fatal outcome, have been reported in patients treated with rituximab. These reports included paraneoplastic pemphigus (an uncommon disorder which is a manifestation of the patient's underlying malignancy), Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. The onset of the reaction in the reported cases has varied from 1-13 weeks following rituximab exposure. Patients experiencing a severe mucocutaneous reaction should not receive any further infusions and seek prompt medical evaluation. Skin biopsy may help to distinguish among different mucocutaneous reactions and guide subsequent treatment. The safety of readministration of rituximab to patients with any of these mucocutaneous reactions has not been determined.

Progressive Multifocal Leukoencephalopathy (PML): John Cunningham (JC) virus infection resulting in PML and death can occur in Rituxan-treated patients with hematologic malignancies or with autoimmune diseases for which Rituxan has not been approved. The majority of patients with hematologic malignancies diagnosed with PML received Rituxan in combination with chemotherapy or as part of a hematopoietic stem cell transplant. The patients with autoimmune diseases had prior or concurrent immunosuppressive therapy and were diagnosed with PML within 12 months of their last infusion of Rituxan.

Consider the diagnosis of PML in any patient presenting with new onset neurologic manifestations. Evaluation of PML includes, but is not limited to, consultation with a neurologist, brain MRI, and lumbar puncture. Discontinue Rituxan and consider discontinuation or reduction of any concomitant chemotherapy or immunosuppressive therapy in patients who develop PML.

Bowel Obstruction and Perforation: Abdominal pain, bowel obstruction and perforation, in some cases leading to death, were observed in patients receiving rituximab in combination with

chemotherapy for DLBCL. In post-marketing reports, which include both patients with low-grade or follicular NHL and DLBCL, the mean time to onset of symptoms was 6 days (range 1-77) in patients with documented gastrointestinal perforation. Complaints of abdominal pain, especially early in the course of treatment, should prompt a thorough diagnostic evaluation and appropriate treatment.

Cardiovascular: The incidence of serious cardiovascular events in the double-blind clinical trial for rheumatoid arthritis (RA) patients was 1.7% and 1.3% in rituximab and placebo groups, respectively. Three cardiovascular deaths occurred during the double-blind period of the RA studies, including all rituximab regimens (3/759 = 0.4%) as compared to none in the placebo group (0/389). Since patients with RA are at increased risk for cardiovascular events compared to the general population, patients with RA should be monitored throughout the infusion and rituximab should be discontinued in the event of a serious or life-threatening cardiac event.

Rituximab infusions should be discontinued in the event of serious or life-threatening cardiac arrhythmias. Patients who develop clinically significant arrhythmias should undergo cardiac monitoring during and after subsequent infusions of rituximab. Patients with pre-existing cardiac conditions including arrhythmias and angina have had recurrences of these events during rituximab therapy and should be monitored throughout the infusion and immediate post-infusion period.

Renal: Rituximab administration has been associated with severe renal toxicity including acute renal failure requiring dialysis and in some cases, has led to a fatal outcome in hematologic malignancy patients. Renal toxicity has occurred in patients with high numbers of circulating malignant cells (> 25,000/mm3) or high tumor burden who experience tumor lysis syndrome and in patients with NHL administered concomitant cisplatin therapy during clinical trials. The combination of cisplatin and rituximab is not an approved treatment regimen. If this combination is used in clinical trials extreme caution should be exercised; patients should be monitored closely for signs of renal failure. Discontinuation of rituximab should be considered for those with rising serum creatinine or oliguria.

Immunization: The safety of immunization with live viral vaccines following rituximab therapy has not been studied and vaccination with live virus vaccines is not recommended. The ability to generate a primary or anamnestic humoral response to vaccination is currently being studies. For patients with NHL, the benefits of primary and/or booster vaccinations should be weighed against the risks of delay in initiation of rituximab therapy.

Carcinogenesis, Impairment of Fertility, Pregnancy, and Nursing: No long-term animal studies have been performed to establish the carcinogenic potential of rituximab. Studies also have not been completed to assess mutagenic potential of rituximab, or to determine potential effects on fertility in males or females. Individuals of childbearing potential should use effective contraceptive methods during treatment and for up to 12 months following rituximab therapy.

It is not known whether rituximab is excreted in human milk. Because human IgG is excreted in human milk and the potential for absorption and immunosuppression in the infant is unknown, women should be advised to discontinue nursing until circulating drug levels are no longer detectable.

The following risks were updated per the Cancer Therapy Evaluation Program's (CTEP), National Cancer Institute (NCI) Action Letter dated June 16th, 2010.

Likely

- Fever
- Reaction that can occur during or following infusion of the drug. The reaction may include fever, chills, rash, low blood pressure, and difficulty breathing.
- Decreased number of a type of white blood cell (lymphocyte)
- Chills

Less Likely

- Lack of enough red blood cells (anemia)
- Thickening of blood/serum as found in Waldenstrom's macroglobulinemia (a cancer of certain blood cells)
- Fever associated with dangerously low levels of a type of white blood cell (neutrophils)
- Heart attack caused by a blockage of a blood vessel supplying part of the heart
- Fast heartbeat; regular rhythm
- Fast heartbeat usually originating in an area located above the ventricles
- Belly Pain
- Diarrhea
- Nausea or the urge to vomit
- Vomiting
- Swelling of the arms and/or legs
- Fatigue or tiredness
- Pain
- Allergic reaction by your body to the drug product that can occur immediately or may be delayed. The reaction may include hives, low blood pressure, wheezing, swelling of the throat and difficulty breathing.
- Infection
- Awakening of viruses which have been latent/dormant
- Infection in HIV positive patients
- Decreased number of a type of white blood cell (neutrophil/granulocyte)
- Decreased number of a type of blood cell that help to clot blood (platelet)
- Decrease in the total number of white blood cells (leukocytes)
- Increased blood sugar level
- Decreased blood level of calcium
- Decreased blood level of potassium
- Joint pain
- Back pain
- Muscle pain
- Pain in the area of the tumor
- Dizziness (or sensation of lightheadedness, unsteadiness, or giddiness)
- Headache or head pain
- Abnormal drowsiness or sluggishness, an unusual lack of energy
- Convulsion or seizure

- Sudden or traumatic injury to the kidney
- Stuffy or runny nose, sneezing
- Sudden constriction of the small airways to the lung that can cause wheezing and shortness of breath
- Cough
- Shortness of breath
- Decrease in the oxygen supply to a tissue
- Inflammation of the lungs that may cause difficulty breathing and can be life threatening
- Sore throat
- Excess sweating
- Itching
- Skin rash with the presence of macules (flat discolored area) and papules (raised bump)
- Swelling of body tissue underneath the skin
- Hives
- Sudden reddening of the face and/or neck
- High blood pressure
- Low blood pressure

Rare, but Serious

Serious, life-threatening allergic reaction requiring immediate medical treatment by your doctor. The reaction may include extremely low blood pressure, swelling of the throat, difficulty breathing, and loss of consciousness.

- Group of signs and symptoms due to rapid breakdown of tumor that can occur after treatment of cancer has started that causes increased levels of blood potassium, uric acid, and phosphate, decreased levels of blood calcium, and kidney failure.
- Disease affecting brain tissue, caused by a virus (specifically the JC virus: Jacob Cruetzfeld virus).
- Severe potentially life-threatening damage to the lungs which can lead to fluid in the lungs.
- Severe reaction of the skin and gut lining that may include rash and shedding or death of tissue.
- Potentially life-threatening condition affecting less than 10% of the skin in which cell death causes the epidermis (outer layer) to separate from the dermis (middle layer).
- Life-threatening condition affecting greater than 30% of the skin in which cell death causes the epidermis (outer layer) to separate from the dermis (middle layer).

Refer to the 'CTEP, NCI Guidelines: Adverse Event Reporting Requirements' http://ctep.cancer.gov/reporting/adeers.html for further clarification.

Note: Rituximab 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.

1.4 Study rationale and selection of drug doses

Synergy

The synergy of rituximab in combination with a purine analog is well described in pre-clinical studies, with randomized prospective clinical trials indicating that the addition of rituximab to fludarabine-based regimens improves OS (Forstpointner et al., 2004). When cladribine was combined with rituximab in a follow-up study of 29 patients with median age of 70, CR was 52%, PR was 14%, median PFS was 12.1 months, and median OS was not reached. Of 15 patients achieving CR, only 3 patients relapsed with the median follow-up of 21.5 months. Patients received a median of 4 cycles. Combination of cladribine and rituximab resulted in 7% grade 3 and 24% grade 4 neutropenia, as well as 14% grade 3 and 3% grade 4 thrombocytopenia. One patient died during treatment from a cerebrovascular incident following a pneumonia. (Inwards et al, 2008). Similar if not more impressive responses were seen at the University of Arizona Cancer Center and Oregon Health and Science University (personal communication, Drs. Miller and Epner).

Robak et al (2006) enrolled 54 patients with mantle cell and indolent lymphomas/CLL in a trial combining rituximab 375 mg/m2 on day 1 with cladribine at 0.12 mg/kg over 2 hrs on days 2-6, with and without cyclophosphamide, on a 28-day cycle. Notably, 42 (78%) patients previously received cladribine. The combination of rituximab and cladribine resulted in 58% PR and 13% CR, with significant improvement in PFS and OS in responders. Of 9 patients with MCL, 2 (22%) had CR and 4 (44%) had PR. Toxicities were hematologic and infectious, including 11% grade 3-4 neutropenia, 7% grade 3-4 thrombocytopenia, 7% grade 3 anemia, 20% grade 3-4 infections (no prophylaxis was used), and 9% pneumonias, including one fatal pneumonia with sepsis in a patient who received rituximab, cladribine, cyclophosphamide. Hypersensitivity reaction with rituximab occurred in 35% of patients.

The synergy of rituximab with bortezomib is described more recently. In vitro data suggests at least additive effect, particularly with rituximab pretreatment in a study CLL cell lines (Smolewski et al., 2006) and in MCL cell lines (Wadehra et al., ASH 2005). Several phase II trials examined combinations of rituximab and bortezomib (given days 1, 4, 8, and 11, on a 3-week cycle). Rituximab, bortezomib, and dexamethasone in relapsed mantle cell lymphoma showed 3 CRs and 6 PRs in 16 evaluable patients (Drach et al., ASH 2007), while in untreated Waldenstrom's macroglobulinemia, this combination resulted in 5 minor and 5 major responses (i.e., CR + PR) in 10 patients (Treon et al., ASH 2006). Phase II study of rituximab and bortezomib in relapsed follicular and marginal zone lymphomas demonstrated 11% CR and 35% PR in 37 evaluable patients (de Vos et al., ASH 2006), while in relapsed Waldenstrom's macroglobulinemia this combination resulted in 7 minimal responses, 3 PRs, and 1 CR, out of 13 patients (Ghobrial et al., ASH 2007). Another phase II trial in relapsed follicular and mantle cell lymphomas and Waldenstrom's macroglobulinemia revealed ORR of 56% in 39 evaluable patients, with FL having ORR of 44%, MCL 46%, and WM 90% (Agathocleous et al., ASH 2007).

Bortezomib has a different mechanism of action, a non-overlapping toxicity profile efficacy in mantle cell and indolent lymphomas, and in vitro synergy with purine analogs (Duechler et al., 2005). An ongoing phase I trial in relapsed/refractory indolent NHL/CLL combines escalating doses of bortezomib on days 1, 4, 8, and 11, with fludarabine, a purine analog, at 25 mg/m2 days 1-3, with addition of rituximab at dose level 5, in a 3-week cycle. Preliminary report showed that at 1.3 mg/m2 of bortezomib DLT has not been reached, but the first patient at dose level 5 developed grade 4 neutropenia. Of 13 patients with median of 3 prior cycles of treatment, 1

achieved CRu (follicular lymphoma) and 1 achieved PR (mantle cell lymphoma), with 6 SD lasting 1.5-3 months and 1 progression (CLL) (Snell et al., ASCO 2006). Our trial will use cladribine on a 4-week cycle, which reflects hematologic recovery with purine analogs better than a 3-week cycle, and will allow time for long-acting growth factor support. The dose of cladribine will be reduced slightly for the combination, similarly to fludarabine-containing combination. The dose of bortezomib will be reduced to days 1 and 4, based on the combination data with R-CHOP (Leonard et al., ASCO 2007) and R-hyperCVAD (Kahl et al., ASCO 2007).

Experience at the University of Arizona Cancer Center includes combination of bortezomib with cladribine and rituximab, given to 5 patients - 13 cycles with bortezomib at 1.6 mg/m2 on day 1, and 4 cycles with bortezomib given at 1.3 mg/m2 on days 1 and 4. Two patients had grade 3 neutropenia and anemia, and one patient had grade 3 thrombocytopenia, with no incidence of neuropathy. Four patients achieved CR, 3 of which are now 6+ months in duration; there have been no hospitalizations (Dr. Miller, personal communication).

Study hypothesis

Our hypothesis is that the combination of bortezomib, cladribine, and rituximab will synergize to produce progression-free survival that is higher than expected from available therapy options in mantle cell and indolent lymphomas.

2 STUDY OBJECTIVES

2.1 Primary Objective

The primary objective of this study is to:

- Determine 2-year PFS in patients with mantle cell, marginal zone, lymphoplasmacytic, small lymphocytic, and relapsed follicular lymphomas
    - Standard definition of PFS will be used: from the time of first drug dose to progression or death from any cause

2.2 Secondary Objectives

The secondary objectives of this study are to:

- Determine 2-year OS;
- Determine CR and ORR;
- Describe long and short-term toxicity;
- Conduct correlative studies:
    - Determine prognostic importance of Aurora kinase A in lymphoma treated with VCR;
    - Determine the cytokine profiles of each lymphoma subtype and how they change with VCR;
    - Evaluate the prognostic importance of major oncogenic pathways in lymphoma treated with VCR using tissue microarray (TMA)

3 INVESTIGATIONAL PLAN

3.1 Overall Design and Plan of the Study

Registration: all patients must be registered centrally by the study coordinator at 520-694-9053 prior to initiation of therapy.

Within 8 weeks of registration:

- Bone marrow biopsy (aspirate not mandatory but suggested as good medical practice)
- Confirmation of pathologic diagnosis (to be performed as standard of care for each study subject enrolled at their respective institutions (The University of Arizona and the University of Massachusetts)
- Pathology block or 10 unstained slides for tissue microarray construction (for correlative studies)

Within 4 weeks of registration:

- CT chest/abdomen/pelvis of diagnostic quality; CT neck optional

Within 2 weeks of registration:

- History and Physical
- Vital signs including weight, height, and performance status
- CBC, comprehensive metabolic panel (includes sodium, potassium, chloride, bicarbonate, urea nitrogen (BUN), creatinine, glucose, total protein, albumin, AST, ALT, alkaline phosphatase, total bilirubin)
- LDH
- Serum Pregnancy test for pre-menopausal women of childbearing age
- Not mandatory but suggested as good medical practice: beta 2-microglobulin and hepatitis B surface antigen
- Serum specimen (5 ml) for cytokine profile (for correlative studies)
- Study registration (Once screening has been complete and evaluated and within 72 hours prior to Day 1, Cycle 1)

Within 2 days of day 1 of cycle 2 ONLY: correlative studies

- Serum specimen (5 ml) for cytokine profile (for correlative studies)
- Optional re-biopsy for tissue microarray (for correlative studies) Pathology block or 10 unstained slides for tissue microarray)

Within 2 days of day 1 of cycles 2-6: routine assessment

- History and Physical
- Vital signs including weight and performance status
- Toxicity assessment
- CBC, comprehensive metabolic panel

Within 7 days of day 1 of cycles 3 and 5 (i.e., after cycles 2 and 4): interim clinical restaging

- CT chest/abdomen/pelvis of diagnostic quality
- LDH (beta 2-microglobulin is not mandatory but suggested as good medical practice)

4-8 weeks after day 1 of cycle 6: full restaging
- History and Physical
- Vital signs including weight and performance status
- Toxicity assessment
- CBC, comprehensive metabolic panel
- LDH (beta 2-microglobulin is not mandatory but suggested as good medical practice)
- CT chest/abdomen/pelvis of diagnostic quality
- Bone marrow biopsy (and aspirate, if performed at baseline) if there was bone marrow involvement on initial biopsy
- Any test performed pre-treatment for staging purposes that was positive for lymphoma (e.g., colonoscopy for mantle cell lymphoma, IgM levels for lymphoplasmacytic lymphoma) should be repeated at restaging.

Every 3 months for 2 years or until progression: clinical restaging
- History and Physical
- Vital signs including weight and performance status
- Toxicity assessment
- CBC, comprehensive metabolic panel
- LDH (beta 2-microglobulin is not mandatory but suggested as good medical practice)
- CT chest/abdomen/pelvis of diagnostic quality

For Waldenstom's macroglobulinemia/lymphoplasmacytic lymphoma only: initial assessment, interim clinical restaging, restaging at therapy completion, and clinical restaging at followup should also include serum protein electrophoresis with immunofixation, IgM level, and serum viscosity.

A study flow chart is provided in section 8.1.

3.2 Selection of Patients

The total number of patients to be registered on this study is 39.
Enrollment is defined as the day of consent; Registration is the day the patient has been determined to be eligible for study and is given a study number.

3.2.1 Inclusion Criteria

Each patient must meet all of the following inclusion criteria to be registered in the study:

- Voluntary written informed consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care.
- Female subject is either post-menopausal or surgically sterilized or willing to use an acceptable method of birth control (i.e., a hormonal contraceptive, intra-uterine device,

diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study.

- Male subject agrees to use an acceptable method for contraception for the duration of the study.
- Biopsy-proven mantle cell, marginal zone, lymphoplasmacytic, small lymphocytic lymphoma, or follicular lymphoma
- CD20-positive disease
- For patients with marginal zone, lymphoplasmacytic, small lymphocytic, or follicular lymphoma – at least one criterion for initiation of treatment must be met:
    - Symptomatic disease
    - Cytopenia related to lymphoma
    - Leukemic phase (> 5,000 malignant lymphocytes/µl)
    - Mass over 5 cm in greatest diameter
    - For lymphoplasmacytic lymphoma: additional treatment criteria are serum viscosity ≥ 4 cp, serum monoclonal protein > 5 g/L, concurrent primary systemic AL amyloidosis, cold agglutinin disease
- Age over 18
- Prior treatment with bortezomib and/or rituximab is acceptable
- For follicular lymphoma only, at least one prior treatment

3.2.2 **Exclusion Criteria**

Patients meeting any of the following exclusion criteria are not to be registered in the study:

- Patient has a platelet count of $< 100 \times 10^9$/L within 14 days before enrollment, unless due to bone marrow infiltration with lymphoma, or due to autoimmune thrombocytopenia because of lymphoma.
- Patient has an absolute neutrophil count of $< 1.0 \times 10^9$/L within 14 days before registration, unless due to bone marrow infiltration with lymphoma.
- Patient has a calculated or measured creatinine clearance of <20 mL/minute within 14 days before registration. (Creatinine Clearance is indicated through the Serum Creatinine. If the Serum Creatinine is abnormal, the physician may then due a 24 hour urine to further clarify Creatinine Clearance. A 24 hour urine test is not required per study.)
- Patient has ≥ Grade 2 peripheral neuropathy within 14 days before registration.
- Myocardial infarction within 6 months prior to registration or has New York Heart Association (NYHA) Class III or IV heart failure (see section 8.4), uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at Screening has to be documented by the investigator as not medically relevant.
- Patient has hypersensitivity to bortezomib, boron or mannitol.

- Female subject is pregnant or breast-feeding. Confirmation that the subject is not pregnant must be established by a negative serum β-human chorionic gonadotropin (β-hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women.
- Patient has received other investigational drugs with 14 days before registration
- Serious medical or psychiatric illness likely to interfere with participation in this clinical study.
- Diagnosed or treated for another malignancy within 3 years of registration, with the exception of complete resection of basal cell carcinoma or squamous cell carcinoma of the skin, an in situ malignancy, or low-risk prostate cancer after curative therapy.
- CNS involvement with lymphoma.
- Known HIV-positive.
- History of disease refractory to a purine analog (defined as remission duration of < 6 months to therapy that included fludarabine, pentostatin, or cladribine).
- History of intolerance of bortezomib, boron, mannitol, cladribine, or rituximab.
- Patient has > 1.5 × ULN Total Bilirubin
- Radiation therapy within 3 weeks before randomization. Enrollment of subjects who require concurrent radiotherapy (which must be localized in its field size) should be deferred until the radiotherapy is completed and 3 weeks have elapsed since the last date of therapy.

3.3 Study Treatments

3.3.1 Clinical Trial Materials

VELCADE (bortezomib) for Injection is a sterile lyophilized powder for reconstitution and is supplied in vials containing VELCADE and mannitol at a 1:10 ratio. For example, vials containing 3.5 mg of VELCADE contain 35 mg of mannitol.

LEUSTATIN (cladribine) for Injection is a clear, colorless, sterile, preservative-free, isotonic solution. It is available in singe-use vials containing 10 mg (1mg/mL) of cladribine. Each milliliter of LEUSTATIN Injection contains 1 mg of the active ingredient and 9 mg (0.15 mEq) of sodium chloride as an inactive ingredient.

Rituxan (rituximab) is a sterile, clear, colorless, preservative-free liquid concentrate for intravenous administration. Rituxan is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 ml) single-use vials. The product is formulated in 9 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dehydrate, 0.7 mg/mL polysorbate 80, and Water for injection.

3.3.2 **Preparation, Handling, and Storage of Drugs**

VELCADE (bortezomib)

Vials containing lyophilized VELCADE for Injection should be stored according to the label requirements. For the United States, store at USP Controlled Room Temperature which is 25ºC (77ºF); excursions permitted from 15 to 30ºC (59 to 86ºF). For Europe, do not store above 30ºC (86ºF). To date, stability data indicate that the lyophilized drug product is stable for at least 18 months when stored under the recommended conditions. Stability studies are ongoing, and Millennium Pharmaceuticals, Inc. will notify the investigator should this information be revised during the conduct of the study.

VELCADE is cytotoxic. As with all cytotoxic drugs, caution is required when preparing and handling VELCADE solutions. Cytotoxic drugs should only be handled by staff specially trained in the safe handling of such preparations. The use of gloves and other appropriate protective clothing is recommended. In case of skin contact, wash the affected area immediately and thoroughly with soap and water for at least 15 minutes. If product contacts eye, immediately flush eye thoroughly with water for at least 15 minutes. Always contact a physician after any form of body contact. All materials that have been used for preparation should be disposed of according to standard practices. A log must be kept of all disposed materials.

Drug is available in sterile, single use vials containing 3.5 mg of VELCADE. Each vial of VELCADE for Injection should be reconstituted under a laminar flow biological cabinet (hood) within eight hours before dosing with 3.5 mL of normal (0.9%) saline, Sodium Chloride Injection USP, so that the reconstituted solution contains VELCADE at a concentration of 1 mg/mL. Prior to reconstitution the vials should remain in the cartons to protect them from light. Dissolution is completed in approximately 10 seconds. The reconstituted solution is clear and colorless, with a final pH of 5 to 6. Reconstituted VELCADE should be administered promptly and in no case more than 8 hours after reconstitution. All materials that have been used for preparation should be disposed of according to standard practices. A log must be kept of all disposed materials.

Leustatin (cladribine)

LEUSTATIN (cladribine) Injection is supplied as a sterile, preservative-free, isotonic solution containing 10 mg (1 mg/mL) of cladribine as 10 mL filled into a single-use clear flint glass 20 mL vial. Leustatin Injection is supplied in 10 mL (1 mg/mL) single-use vials (NDC 59676-201-01) available in a treatment set (case) of seven vials. Store refrigerated 2º to 8ºC (36º to 46ºF). Protect from light during storage.

When stored in refrigerated conditions at 2º-8ºC (36º-46ºF) protected from sunlight, unopened vials of Leustatin Injection are stable until the expiration date indicated on the package. Freezing does not adversely affect the solution. If freezing occurs, thaw naturally to room temperature. DO NOT heat or microwave. Once thawed, the vial of Leustatin Injection is stable until the expiration date if refrigerated. DO NOT refreeze. Once diluted, solutions containing Leustatin Injection should be administered promptly or stored in the refrigerator (2º-8ºC) for no more than 8 hours prior to administration

Cladribine is a cytotoxic agent. The potential hazards associated with cytotoxic agents are well established and proper precautions should be taken when handling, preparing, and administering Leustatin Injection. The use of disposable gloves and protective garments is recommended. If Leustatin Injection contacts the skin or mucous membranes, wash the involved surface immediately with copious amounts of water. Several guidelines on this subject have been published. There is no general agreement that all of the procedures recommended in the guidelines are necessary or appropriate. All materials that have been used for preparation should be disposed of according to standard practices. A log must be kept of all disposed materials..

Administration: Add the calculated dose of Leustatin Injection to an infusion bag containing 500 mL of 0.9% Sodium Chloride Injection, USP. The use of 5% dextrose as a diluent is not recommended because of increased degradation of cladribine. Admixtures of Leustatin Injection are chemically and physically stable for at least 24 hours at room temperature under normal room fluorescent light in Baxter Viaflex® PVC infusion containers. Since limited compatibility data are available, adherence to the recommended diluents and infusion systems is advised. Solutions containing Leustatin Injection should not be mixed with other intravenous drugs or additives or infused simultaneously via a common intravenous line, since compatibility testing has not been performed.

Once diluted, solutions of Leustatin Injection should be administered promptly or stored in the refrigerator (2º to 8ºC) for no more than 8 hours prior to start of administration. Vials of Leustatin Injection are for single-use only.

Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration, whenever solution and container permit. A precipitate may occur during the exposure of Leustatin Injection to low temperatures; it may be resolubilized by allowing the solution to warm naturally to room temperature and by shaking vigorously. DO NOT HEAT OR MICROWAVE.

Rituxan (rituximab)

Formulation: Rituximab antibody will be provided in 100 mg (10 mL) and 500 mg (50 ml) pharmaceutical grade vials at a concentration of 10 mg of protein per mL (actual concentration should be noted on the product label).

Storage and Stability: Rituximab should be stored at 2 - 8°C. Do not freeze or store at room temperature. The product is a protein- HANDLE GENTLY AND AVOID FOAMING. The avoidance of foaming during product handling, preparation and administration is important, as foaming may lead to the de-naturing of the product proteins.

Prepare the rituximab infusion solution as follows:

1. If a delay in administration of the infusion occurs after the product is prepared, the properly identified container may be kept refrigerated at 2 - 8°C for up to six hours.
2. Use sterile, non-pyrogenic, disposable containers, syringes, needles, stopcocks and transfer tubing, etc.

3. Transfer of the rituximab from the glass vial should be made by using a suitable sterile graduated syringe and large gauge needle.
4. Transfer the appropriate amount of rituximab from the graduated syringe, into a partially filled IV pack containing sterile, pyrogen-free 0.9% sodium chloride solution, USP (saline solution). The final concentration of rituximab in saline solution should be a maximum of 1 mg/ml. Mix by inverting the bag gently. DO NOT USE A VACUUM APPARATUS to transfer the product from the syringe to the plastic bag.
5. Place an IV administration set into the outflow port of the bag containing the infusion solution.
6. NOTE: DO NOT USE evacuated glass containers which require vented administration sets because this causes foaming as air bubbles pass through the solution.

The administration of rituximab will be accomplished by slow IV infusion. CAUTION: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS. IV pumps such as the IMED 960 may be used with the rituximab infusion. DO NOT INFUSE CONCOMITANTLY with another IV solution or IV medications. Prime the line with the rituximab solution such that approximately 30 mL are delivered. This will saturate the filter and tubing.

Drug administration and dosage schedule

Combination of bortezomib, cladribine, and rituximab will be administered as follows:

- Rituximab 375 mg/m2 IV day 1
- Cladribine 4 mg/m2 IV over 2 hours days 1-5
- Bortezomib 1.3 mg/m2 IV days 1 and 4

The combination will be repeated every 28 days, which would constitute a cycle, for a maximum of 6 cycles

VELCADE Administration

VELCADE will be administered at 1.3 mg/m2 on days 1 and 4 of each cycle.

Drug will be administered only to eligible patients under the supervision of the investigator or identified sub-investigator(s). Patients may be treated on an out-patient basis, if possible. The pharmacist will prepare the drug under aseptic conditions. The amount (in mg) of drug to be administered will be determined based on body surface area. Body surface area is to be calculated based on body weight using a standard nomogram (see section 8.3). The dose should be calculated on Day 1 of each cycle; the dose administered should remain the same throughout each cycle but should be recalculated at the start of the next cycle. If a patient experiences a notable change in weight (e.g., loss or gain of ≥8 lbs or 3.6 kg) within a cycle, as determined by an unscheduled weight assessment, then the patient's dose should be recalculated at that time.
The appropriate amount of VELCADE will be drawn from the injection vial and administered as an intravenous (IV) push over 3 to 5 seconds followed by a standard saline flush or through a running IV line. Vials are for single use administration.

VELCADE Destruction

For commercially-labeled VELCADE for IND-exempt studies, please contact your Millennium Clinical Operations representative to arrange for return of study drug procedures. Any unused or expired VELCADE must be returned to Millennium. Be sure to document drug return on your drug accountability logs.

Leustatin (cladribine) administration

Cladribine will be administered at 4 mg/m^2 on days 1 through 5 of each cycle as a 2-hour intravenous infusion. Vials of Leustatin Injection are for single-use only. Drug will be administered only to eligible patients under the supervision of the investigator or identified sub-investigator(s). Patients may be treated on an out-patient basis, if possible.

The pharmacist will prepare the drug under aseptic conditions. The amount (in mg) of drug to be administered will be determined based on body surface area. Body surface area is to be calculated based on body weight using a standard nomogram (see section 8.3). The dose should be calculated on Day 1 of each cycle and should be adjusted if there is at least 10% change in weight.

Rituxan (rituximab) Administration

Rituximab will be administered at 375 mg/m^2 as a slow intravenous infusion on day 1 of each cycle. Drug will be administered only to eligible patients under the supervision of the investigator or identified sub-investigator(s). Patients may be treated on an out-patient basis, if possible. The pharmacist will prepare the drug under aseptic conditions. The amount (in mg) of drug to be administered will be determined based on body surface area. Body surface area is to be calculated based on body weight using a standard nomogram (see section 8.3). The dose should be calculated on Day 1 of each cycle and should be adjusted if there is at least 10% change in weight.

3.3.4 Dose Modification and Delay

Dose escalation will not be allowed in any patient, and there must be at least 72 hours between each dose of VELCADE.

Before each cycle, the patient will be evaluated for possible toxicities that may have occurred after the previous cycle(s). Toxicities are to be assessed according to the NCI Common Terminology Criteria for Adverse Events (CTCAE), Version 3.0.

Dose level reductions are as follows:
VELCADE:
Dose level 0: 1.3 mg/m^2 days 1 and 4 (baseline)
Dose level 1: 1.0 mg days 1 and 4

Dose Level 2: 0.7 mg days 1 and 4

Cladribine:
Dose level 0: 4 mg/m2 days 1-5 (baseline)
Dose level 1: 4 mg/m2 days 1-4

Rituximab: no dose level reductions.
Hematologic toxicities:

After the first cycle of combination chemotherapy, the subsequent cycle is to be held for up to 2 weeks (14 days) until the patient has a platelet value of at least 75×10^9/L and absolute neutrophil count of at least 1.0×10^9/L. If the platelet value does not recover by the 14 days post the subsequent cycle, then the patient will be removed from the trial.

Due to high risk of neutropenia with this regimen, patients should be started on granulocyte growth factors prophylactically. Growth factors for erythroid and megakaryocytic lineages are to be used as needed.

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

Thrombocytopenia:

Grade 1 ($\geq 75 \times 10^9$/L) – proceed with therapy; no dose reduction
Grade 2 (50-75 $\times 10^9$/L) – hold therapy until count recovery to grade 0-1; no dose reduction; rule out factors potentially contributing to thrombocytopenia (such as platelet-toxic drugs);
Grades 3-4 ($< 50 \times 10^9$/L) – 1st episode: hold therapy until recovery to grade 0-1; rule out factors potentially contributing to thrombocytopenia (such as platelet-toxic drugs); decrease cladribine by 1 dose level for > 1 week delay;
2nd episode: hold therapy until recovery to grade 0-1; decrease VELCADE by 1 dose level for > 1 week delay;
3rd episode: discontinue the protocol.

Neutropenia:

Grades 1-2 (ANC $\geq 1.0 \times 10^9$/L) – proceed with therapy; no dose reduction; increase growth factor support if applicable;

Grades 3-4 (ANC $< 1.0 \times 10^9$/L) – hold therapy until recovery to grade 0-2; increase growth factor support if applicable; decrease cladribine by 1 dose level for > 1 week delay. If no more dose reductions possible, discontinue the protocol.

Anemia:

Grades 1-2 (Hgb ≥ 8 g/dL) – proceed with therapy; no dose reduction; use appropriate supportive measures (such as erythropoiesis supporting agents and transfusions);

Grades 3-4 (Hgb < 8 g/dL) – hold therapy until recovery to grade 0-2; no dose reduction; use appropriate supportive measures (such as erythropoiesis supporting agents and transfusions). If recovery time is more 2 weeks, discontinue the protocol.

Non-hematologic toxicities (except neuropathic pain and/or peripheral sensory neuropathy):

VELCADE dose modification:

If the patient experiences any ≥ Grade 3 non-hematologic toxicity considered by the investigator to be related to VELCADE, then therapy is to be held.

VELCADE is to be held for up to 2 weeks until the toxicity returns to Grade 1 or better. If, after therapy has been held, the toxicity does not resolve, as defined above, then the protocol must be discontinued.

If the toxicity resolves, as defined above, and VELCADE is to be restarted, the dose must be reduced by one dose level.

Cladribine dose modification:

If the patient experiences any ≥Grade 3 non-hematologic toxicity considered by the investigator to be related to cladribine, then therapy is to be held.

Particular attention needs to be paid to renal and neurological toxicities.

Cladribine is to be held for up to 2 weeks until the toxicity returns to Grade 1 or better. If, after therapy has been held, the toxicity does not resolve, as defined above, then the protocol must be discontinued.

If the toxicity resolves, as defined above, and cladribine is to be restarted, the dose must be reduced by one dose level.

Rituximab Dose Antibody Modification:

a. Patients may experience transient fever and rigors with infusion of rituximab. If Grade 3 fever (or Grade 2 fever with rigors) or Grade 2 rigors are noted, the antibody infusion should be temporarily discontinued, the patient should be observed, and the severity of the side effects should be evaluated. The patient should be treated according to the best available local practices and procedures. Following observation, when fever resolves to Grade 2 or less and rigors to Grade 1 or less, the infusion should be continued, initially, at 1/2 the previous rate. Following the antibody infusion, the IV line should be kept open for medications, as needed.

b. Hypotension, bronchospasm and angioedema have occurred as part of an infusion related symptom complex. If a Grade 3 or greater hypersensitivity/allergic reaction occurs, antibody infusion should be interrupted and may be resumed at a 50% reduction in rate when symptoms have completely resolved. Treatment with diphenhydramine and acetaminophen is recommended; additional treatment with bronchodilators or IV saline may be used at the physician's discretion.

c. Precautionary hospitalization for patients experiencing severe infusion symptoms, which do not resolve after discontinuation of the cycle, is recommended. If there are no complications, the IV line may be discontinued after one hour of observation. If complications occur during the rituximab infusion, the patient should be observed for two hours after the completion of the infusion. If a patient experiences a Grade 3 toxicity that persists until the next scheduled infusion, the patient must discontinue treatment until toxicities have resolved to Grade 2 or less. If treatment is delayed for more than three weeks, remove the patient from protocol treatment.

d. Tumor Lysis Syndrome: Appropriate medical therapy should be provided for patients who develop tumor lysis syndrome. Following treatment for and resolution of tumor lysis syndrome, subsequent rituximab therapy may be administered in conjunction with prophylactic therapy for this syndrome. Contact the Study Coordinator prior to resuming treatment in these patients.

e. Hepatitis B Reactivation with Related Fulminant Hepatitis and Other Viral Infections: Carriers of hepatitis B should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis throughout their study participation. Patients with any evidence of active hepatic disease or known HBV infection should be managed as clinically appropriate and should only receive rituximab if they have control of the infection and are adequately informed of the risks. Patients who have never received vaccination for HBV, and have not had serologic testing for HBsAg, should be tested for surface antigen positivity.

   In patients who develop progressive multifocal leukoencephalopathy (PML), rituximab should be discontinued and reductions or discontinuation of concomitant immunosuppressive therapy and appropriate treatment, including antiviral therapy, should be considered. Physicians should consider PML in any patients presenting with new onset neurologic manifestations, particularly in patients with systemic lupus erythematosus (SLE) or lymphoid malignancies. Consultation with a neurologist, brain MRI, and lumbar puncture should be

considered as clinically indicated. There are no known interventions that can reliably prevent PML or adequately treat PML if it occurs.

f. Severe Mucocutaneous Reactions: All patients on and off rituximab therapy should be closely monitored for signs and symptoms suggestive of severe cutaneous and mucocutaneous reactions. Should these symptoms arise, discontinue rituximab therapy (if applicable) and support as clinically indicated.

g. Cardiovascular events: Patients with rheumatoid arthritis (RA) are at increased risk for cardiovascular events compared to the general population. Patients with RA should be monitored throughout the infusion, and rituximab should be discontinued in the event of a serious or life-threatening cardiac event.

   Patients who develop clinically significant arrhythmias should undergo cardiac monitoring during and after subsequent infusions of rituximab. Patients with pre-existing cardiac conditions, including arrhythmias and angina, that have had recurrences of these events during rituximab therapy should be monitored throughout the infusion and immediate post-infusion period. Patients off rituximab therapy should be closely monitored for signs and symptoms suggestive of life-threatening cardiac events and supported as clinically indicated.

h. Bowel obstruction and perforation: Complaints of abdominal pain, especially early in the course, should prompt a thorough diagnostic evaluation and appropriate treatment. If patient experiences a bowel obstruction or perforation, discontinue rituximab therapy. Patients off rituximab therapy should be closely monitored for signs and symptoms suggestive of bowel obstruction and supported as clinically indicated.

i. Renal: Discontinuation of rituximab should be considered for those with rising serum creatinine or oliguria.

Neuropathic pain and/or peripheral sensory neuropathy:

Patients who experience VELCADE-related neuropathic pain and/or peripheral sensory neuropathy are to be managed as presented in Table 4 Management of Patients with VELCADE-Related Neuropathic Pain and/or Peripheral Sensory Neuropathy.

Table 4

Management of Patients with VELCADE Related Neuropathic Pain and/or Peripheral Sensory or Motor Neuropathy

Recommended Dose Modification for VELCADE related Neuropathic Pain and/or Peripheral Sensory or Motor Neuropathy	
Severity of Peripheral Neuropathy Signs and Symptoms	**Modification of Dose and Regimen**
Grade 1 (paresthesias, weakness and/or loss of reflexes) without pain or loss of function	No action
Grade 1 with pain or Grade 2 (interfering with function but not with activities of daily living)	Reduce VELCADE to 1.0 mg/m^2
Grade 2 with pain or Grade 3 (interfering with activities of daily living)	Withhold* VELCADE therapy until toxicity resolves. When toxicity resolves reinitiate with a reduced dose of VELCADE at 0.7 mg/m^2.*
Grade 4 (Sensory neuropathy which is disabling or motor neuropathy that is life threatening or leads to paralysis)	Discontinue VELCADE
Grading based on NCI Common Terminology Criteria CTCAE v3.0 NCI Common Terminology Criteria website - http://ctep.info.nih.gov/reporting/ctc.html	

ADL = activities of daily living

*Key:

Reduce by one dose level:	VELCADE dose reduction from 1.3 to 1.0, or 1.0 to 0.7 mg/m^2/dose.
Reduce by two dose levels:	VELCADE dose reduction from 1.3 or 1.0 to 0.7 mg/m^2/dose.
Hold:	Interrupt VELCADE for up to 2 weeks until the toxicity returns to Grade 1 or better.

The neurotoxicity-directed questionnaire (see section 8.6) is a useful tool for determining the presence and intensity of neuropathic pain and/or peripheral neuropathy from the patient's perspective. Neuropathic symptoms are more prominent than abnormalities on the clinical examination. After the patient completes the neurotoxicity-directed questionnaire, the questionnaire should be reviewed to assist with the evaluation of the onset and intensity of peripheral neuropathy and other neurotoxicities that may possibly require intervention or dose modification.

Patients with mild hepatic impairment (bilirubin $\leq 1.5 \times$ ULN) do not require a starting dose adjustment. Please note that patients with bilirubin levels > 1.5 ULN are excluded from enrollment in this protocol. If a patient develops moderate or severe hepatic impairment with bilirubin $\geq$ Grade 2 (> 1.5 -3.0 X ULN) while on study, the investigator should hold VELCADE until the toxicity returns to $<$ Grade 2. Restarting VELCADE at the next lower dosed level could be considered at the Investigator's discretion and following exclusion of VELCADE-induced liver impairment and careful consideration of liver disease due to other causes, such as, but not limited to, active infection and multiple myeloma-related liver disease.

3.3.5 Treatment Assignment

This is an open-label trial without randomization. All patients will be assigned to the same treatment.

3.3.6 Blinding, Packaging, and Labeling

VELCADE will be supplied by Millennium Pharmaceuticals, Inc, in vials as open-label stock. Both the box label and vial label will fulfill all requirements specified by governing regulations.

Cladribine and rituximab are commercially available and will be labeled as specified by governing regulations.

3.3.7 Concomitant Treatment

Investigators should consider using antiviral prophylaxis in subjects being treated with VELCADE.

Required Concurrent Therapy

The following medications/supportive therapies are required during study participation, as applicable:

- Due to high likelihood of neutropenia with cladribine, prophylactic use of Neupogen, Neulasta, or Leukine is mandatory
- Prophylactic antibiotic therapy to prevent febrile neutropenia is at the discretion of the treating physician (e.g., ciprofloxacin, levofloxacin)
- *Pneumocystis jirovecii (carinii)* pneumonia prophylaxis is mandatory; exact agent is per discretion of the treating physician (trimethoprim/sulfamethoxazole is preferred)
- Anti-herpetic prophylaxis is mandatory; exact agent is per discretion of the treating physician (e.g., acyclovir, valacyclovir, or famcyclovir)
- Anti-fungal prophylaxis is not mandatory but is strongly encouraged; exact agent is per discretion of the treating physician (e.g., fluconazole)
- Pre-medication prior to rituximab infusion is mandatory and is per institutional guidelines
- To prevent tumor lysis syndrome in patients with bulky tumors or significant bone marrow or blood involvement, oral or IV fluid intake in excess of 2 L daily is encouraged during therapy. Patients must receive allopurinol, dose-adjusted for renal clearance, for the first two weeks of the first cycle of protocol therapy. Allopurinol may be continued after the first two weeks at physician's discretion.
- Growth factors for erythroid and megakaryocytic lineages are to be given at the discretion of the treating physician.

Prohibited Concurrent Therapy

- Any investigational agent other than VELCADE, cladribine, and rituximab, within 14 days of the start of this trial and throughout the duration of this trial.

3.3.8 Treatment Compliance

All drug will be administered to eligible patients under the supervision of the investigator or identified sub-investigator(s). The pharmacist will maintain records of drug receipt (if applicable), drug preparation, and dispensing, including the applicable lot numbers, patients' height, body weight, and body surface area (see section 8.3), and total drug administered in milliliters and milligrams, and date and time of administration. Any discrepancy between the calculated dose and dose administered and the reason for the discrepancy must be recorded in the source documents.

3.4 Duration of Treatment and Patient Participation

Patients will receive a maximum of 6 treatment cycles. They will have short-term follow-up every 3 months for two years. Patients will have long-term follow-up phone calls annually up to 5 years..

3.5 Termination of Treatment and/or Study Participation

Patients will be informed that they have the right to withdraw from the study at any time for any reason, without prejudice to their medical care. The investigator also has the right to withdraw patients from the study for any of the following reasons:

- Intercurrent illness
- Occurrence of an unacceptable adverse event
- A treatment cycle delay of more than 2 weeks consecutively
- Patient request
- Protocol violations
- Non-compliance
- Administrative reasons
- Failure to return for follow-up
- General or specific changes in the patient's condition unacceptable for further treatment in the judgment of the investigator
- Progressive disease at any time

At the time of withdrawal, all study procedures outlined for the End of Study visit should be completed. The primary reason for a patient's withdrawal from the study is to be recorded in the source documents.

3.6 Efficacy, Pharmacodynamic/Pharmacogenomic/Correlative studies, and Safety Measurements

3.6.1 Correlative Studies

Specific Aim 1: To investigate human Mantle Cell Lymphoma, and Indolent Lymphoma utilizing a tissue microarray (TMA) to demonstrate over-expression of Aurora A, Aurora B, Ki-67, cyclin D, Bcl-2, phosphor-HisH3, c-Met and VEGF with RT-PCR validation.

Overview:

Tissue microarray (TMA) is a powerful tool to perform high-throughput evaluation of cell phenotypes and specific tumor-associated antigens when integrated with immunohistochemical

(IHC) staining or *in situ* hybridization (Wan et al., 1987; Kononen et al., 1998). Validation studies using tumor specimens from different cancers and lymphoma have demonstrated that TMAs can accurately represent the IHC staining results of whole tissue or lymph node sections when multiple cores are used (Skacel et al., 2002; Rassidakis et al., 2002). Moreover, this technique facilitates the evaluation of new biomarkers for disease diagnosis and prognosis (Natkunam et al, 2001). TMA phenotypic studies of Hodgkin's lymphomas and B-cell non-Hodgkin's lymphomas are reported but the extent of potential variability among observers and methods from different laboratories needs further characterization. Recent studies in B-cell NHL suggest that tumor antigen markers may be used as surrogates for the gene expression profile with equivalent prognostic significance (Natkunam et al, 2001; Zu et al, 2005). Further, we would like to confirm this at the transcript level by RNA isolation and quantitative real time RT-PCR. The proposed TMA will be on tissue retrospectively and/or prospectively collected and the information derived will be utilized as surrogate markers and validated in the planned Phase II trial. We plan to focus on the hallmarks of cancer known to lymphoma biology: Aurora kinase A, Aurora kinase B, Ki-67, cyclin D, Bcl-2, phosphor-HisH3, c-Met and VEGF. These markers can also be validated by Western blotting (if the TMA turns out to be non-conclusive).

Aurora kinase A, a serine/threonine kinase, is involved in regulating centrosome assembly and chromosome segregation during mitosis. Aurora kinase A is known to be overexpressed in several NHL subtypes (Yakushijin et al., 2004) and may be related to proliferation status in MCL (Camacho et al., 2006). It is thus our hypothesis that Aurora Kinase A is responsible for a large portion of the effect of proliferative signature that is most prognostic in mantle cell lymphoma as assessed by LLMPP (data from Rosenwald et al, 2003, reviewed by Dr. Rimsza, personal communication). Aurora kinase B may be similarly significant.

Tissue Array Construction and Immunohistochemistry

Morphologically representative areas of all cases of lymphoma on this study will be evaluated. Also 3 to 4 normal 'reactive' lymph nodes will be used as controls. In each case, 3 cores with a diameter of 0.6mm will be obtained from 3 different areas of the tissue blocks and re-embedded in a tissue microarray paraffin block using a tissue arrayer (Beecher Instruments, Silver Spring, MD) according to a method described previously (Kononen et al., 1998). Serial sections of the paraffin-embedded lymphoma tissue array will be deparaffinized and reacted with primary IHC antibody specific to markers listed above Before antibody incubation, the slides will be processed for antigen retrieval. This will consist of microwaving the slides in citrate buffer (0.1 M, pH 6.0) in a pressure cooker for 25 min and then left to cool. The slides will be incubated with the antibody for 1 hour. Biotinylated antimouse/antirabbit secondary antibodies will be applied, followed by streptavidin-peroxidase complex (DAKO, Carpinteria, CA). Colored products will be produced using the diaminobenzidine substrate. Staining reactions will be scored as diffuse or focal and graded (from 0, negative to 4+, intensely positive) for both neoplasm and background stroma.

RNA Isolation and RT-PCR

Total RNA will be extracted from all aggressive B-cell NHL samples utilizing the RNeasy Mini Kit (Qiagen, CA). The amount of total RNA isolated from the cells will be quantified using spectrophotometric OD_{260} measurements with yields > 25 µg/sample. Control RNA will be obtained from normal peripheral B-cells from 4 volunteers (AllCells, CA) and three snap frozen

normal lymph node (University of Arizona, Department of Pathology). One hundred nanograms of total RNA will be used for reverse transcriptase (RT) reactions (20 ml total volume) carried out using SuperScript™ III Platinum® Reverse Transcriptase (Invitrogen, Carlsbad, CA). Reactions will be incubated at 42^{0}C for 50 minutes followed by incubation at 37^{0}C with RNase H for 20 minutes. An Opticon DNA Engine (MJ Research, Reno, NV) will be used to perform real-time fluorescence detection PCR. One microliter of cDNA produced from reverse transcription reactions was added to 12.5 µl of Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen, Carlsbad, CA), 1 µl of gene-specific or β-actin specific primer pair (see below for primer design), and 10.5 µl of dH2O (final volume of 25 µl). Amplification (95^{0}C for 15 s, 55^{0}C for 30 s, and 72^{0}C for 30 s) will be repeated for 44 cycles. Following the PCR reaction, a melting curve assay was performed to determine the purity of the amplified product. Data were provided as a threshold cycle value (C_t) for each sample which indicated the cycle at which a statistically significant increase in fluorescence was first detected. These data were then normalized to β-actin, which serves as an unaffected control gene, for each data point and compared to normal B-cell controls to determine relative expression ratios. Each measurement was performed in triplicate and results represented as mean $\pm$ SD.

Primer Design

PCR primers for above genes will be designed using MacVector® (Accelrys, San Diego, CA) to produce amplicons with lengths ranging from 80-250 bp to optimize the efficiency of qPCR. β-actin primers from QuantumRNA™ β-Actin Internal Standards (Ambion, Austin, TX) will be used to normalize the qPCR data.

Specific Aim 2: To investigate the serum cytokine profile prior to and post therapy for identification of predictive and potential prognostic markers of response.

Overview

Cytokine expression in patients with lymphoma is dysregulated in a subtype-specific way (Airoldi et al., 2001). We will assess the cytokine profile of all lymphoma subtypes pre-treatment and after 1 cycle of therapy. The cytokine profile will likely differ by lymphoma subtype, may differ in responders as compared to non-responders, and may change with treatment in each lymphoma subtype in a unique way that would reveal underlying biologic differences. In particular, we will assess plasma TNF-alpha, the reduction in which was associated with response to bortezomib in relapsed lymphomas (Strauss et al., 2006). Other cytokines that have evidence of significance will be explored, particularly from TNF and TGF-beta families, as well as IL-10 and IL-6.

Serum Cytokine Profiling

Stored (-80ºC) or fresh patient serum samples will be utilized (N= all patients and 4 normal volunteers). Array slides will be thawed at room temperature as serum was diluted 3.5-fold with 1x blocking buffer (Cytokine Array Protocol, RayBiotech, Inc #AAH-CYT-G1000). Slides will be blocked with blocking buffer and 50µL of each sample was hybridized with the slide for 2 hours; 1µL internal controls will also be added. After washing, provided biotin-conjugated anti-cytokine antibodies will be diluted with 300µL of blocking buffer, added to the slide, and

incubated for 2 hours at room temperature. Washing steps will be repeated, followed by addition of a 1:1500 dilution of Alexa Flour 555-conjugated streptavidin and incubated overnight at 4°C. Slides will be washed multiple times and read on the Axon GenePix using the cy3 channel. Analysis will be conducted using the RayBiotech analysis tool. Results will be represented as mean± SE as each sample per cytokine has 3 data points.

Special Instructions for Sample Acquisition for Corollary Studies

Tumor:

1. Paraffin-embedded pre-treatment specimen from the most recent performed (within 8 weeks of study registration) tumor biopsy. 10 unstained slides or tissue block will need to be submitted.

2. Paraffin-embedded OPTIONAL tumor biopsy specimen (collected within 2 days of Cycle 2, day 1. 10 unstained slides or tissue block will need to be submitted.

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Blood: Peripheral serum sample:
Serum samples (5 ml serum) are to be collected within 2 weeks of study registration and within 2 days of cycle 2, day 1 (prior to treatment infusion). For the U Mass site, each serum sample will be divided into 1 ml aliquot cryovials and placed in a minus 80° C. freezer for future batched shipment. For UA site, submit red top serum separator tube at room temperature to Dr. Rimsza's lab (per appendix 8.11) within 2 hours of the blood collection.

Sample Acquisition Calendar for Corollary Studies

SAMPLE	PRE-STUDY	WITHIN 2 DAYS OF CYCLE 2, DAY 1
TUMOR[1]	X	X
BLOOD[2]	X	X

[1] Tumor samples: Paraffin-embedded pretreatment block or 10 unstained slides (from most recent biopsy procedure collected within 8 weeks of study registration) and 1 paraffin embedded block or 10 unstained slides from OPTIONAL tissue biopsy to be performed within 2 days of Cycle 2, day 1.

[2] Blood samples: Serum samples (5 ml serum) are to be collected within 2 weeks of study registration and within 2 days of cycle 2, day 1 (prior to treatment infusion).

Shipping and Handling of Study Specimens Instructions

Samples will only be received Monday-Friday (excluding holidays) between 8:00 am and 2:00 pm MST.

A Tissue Submission Form (See APPENDIX 8.11) must be completed for each set of samples collected for an individual subject.

All submitted specimens must be labeled accordingly:

1. Type of sample (serum,)
2. Time point of collection (eg., Cycle 2 day 1)
3. Date of specimen collection
4. Actual time of specimen collection
5. Protocol ID (UACC/U Mass)
6. Subject's initials
7. Subject's study number

The Federal guidelines for shipment are as follows:

1. The specimen must be wrapped in an absorbable material.
2. The specimen must be placed in an AIRTIGHT container (like a re-sealable bag).
3. Pack the re-sealable bag and specimen in a Styrofoam shipping container
4. Pack the Styrofoam shipping container in a cardboard box.
5. The cardboard box must be marked as "BIOHAZARD".

Shipment of paraffin blocks and slides require the inclusion of a cold pack when sending materials to Arizona.

Shipment of processed draw tubes (serum) require shipment on dry ice which requires a dry ice label on the shipping container and a MINIMUM of 5 to 10 pounds of dry ice.

UMass samples may be batched. Contact Betty Glinsmann-Gibson at the Lymphoma SPORE Tissue Service before you intend to ship any specimens to assure proper receipt of the specimens.

Send to:
Betty Glinsmann-Gibson, MS
Research Laboratory of Lisa Rimsza, MD
University of Arizona,
Department of Pathology
AHSC room 5208
1501 N. Campbell Avenue
Tucson AZ 85724-5043

bjglinsm@email.arizona.edu
Phone: 520-626-7894
Fax: 520-626-6081

4 ADVERSE EVENTS

4.1 Definitions

All serious adverse events (SAEs) (regardless of expectedness, causality, and whether commercial or investigational VELCADE is used) must be reported to Millennium Pharmacovigilance (or designee). See Section 4.2 for the reporting of SAEs.

The sponsor-investigator is responsible to meet all regulations and requirements applicable to the sponsor-investigator.

4.1.1 Adverse Event Definition

An **adverse event** (AE) is any untoward medical occurrence in a patient administered a pharmaceutical product, which does not necessarily have a causal relationship with the treatment. An adverse event can be any unfavorable and unintended sign (e.g., including an abnormal laboratory finding), symptom, or disease temporally associated with the use of the drug, whether or not it is considered to be drug related. This includes any newly occurring event or previous condition that has increased in severity or frequency since the administration of drug.

For this protocol an abnormal laboratory value will not be assessed as an AE unless that value leads to discontinuation or delay in treatment, dose modification, therapeutic intervention, or is considered by the investigator to be a clinically significant change from baseline.

4.1.2 Serious Adverse Event Definition

A **serious adverse event** (SAE) is any adverse event, occurring at any dose and regardless of causality that:

- Results in **death**.
- Is **life-threatening**. Life-threatening means that the patient was at immediate risk of death from the reaction as it occurred, i.e., it does not include a reaction which hypothetically might have caused death had it occurred in a more severe form.
- Requires inpatient **hospitalization or prolongation of existing hospitalization**. Hospitalization admissions and/or surgical operations scheduled to occur during the study period, but planned prior to study entry are not considered AEs if the illness or disease existed before the patient was registered in the trial, provided that it did not deteriorate in an unexpected manner during the trial (e.g., surgery performed earlier than planned).
- Results in **persistent or significant incapacity** or substantial disruption of the ability to conduct normal life functions.
- Is a congenital anomaly/birth defect.
- Is an **important medical event**. An important medical event is an event that may not result in death, be life-threatening, or require hospitalization but may be considered an SAE when, based upon appropriate medical judgment, it may jeopardize the patient or subject and may require medical or surgical intervention to prevent one of the outcomes listed in the definitions for SAEs. Examples of such medical events include allergic bronchospasm requiring intensive treatment in an emergency room or at home, blood

dyscrasias or convulsions that do not result in inpatient hospitalization, or the development of drug dependency or drug abuse.

Clarification should be made between the terms "serious" and "severe" since they ARE NOT synonymous. The term "severe" is often used to describe the intensity (severity) of a specific event (as in mild, moderate, or severe myocardial infarction); the event itself, however, may be of relatively minor medical significance (such as a severe headache). This is NOT the same as "serious," which is based on patient/event outcome or action criteria described above and are usually associated with events that pose a threat to a patient's life or functioning. A severe adverse event does not necessarily need to be considered serious. For example, persistent nausea of several hours duration may be considered severe nausea but not an SAE. On the other hand, a stroke resulting in only a minor degree of disability may be considered mild, but would be defined as an SAE based on the above noted criteria. Seriousness (not severity) serves as a guide for defining regulatory reporting obligations.

4.2 Procedures for AE and SAE Reporting

Adverse event recording will begin on Day 1 of the first treatment cycle, and will continue until 30 days after the subject receives the last dose of study drug (s).

All AEs will be entered in the Oncore database system for subjects enrolled at the University of Arizona Cancer Center (UACC). All adverse events for each subject enrolled at the University of Massachusetts Memorial Cancer Center will be entered in the UACC Oncore database remotely by designated study staff from the University of Massachusetts Memorial Cancer Center. Adverse event data will be shared between the UACC and the University of Massachusetts Memorial Cancer Center at least quarterly. The Study Coordinator or Research Nurse from each institution will print out the adverse event data capture form in Oncore and scan the document to the Principal Investigator at each participating center.

Serious adverse event (SAE) recording will begin on Day 1 of the first treatment cycle, and will continue until 30 days after the subject receives the last dose of study drug (s). All serious adverse events (SAEs) that occur at the University of Arizona Cancer Center or the University of Massachusetts Memorial Cancer Center must be reported to the principal investigator and sponsor/investigator, Daniel Persky, at 520-626-8908, within 24 hours of notification of the event, using a study specific SAE form.

The study specific SAE CRF (provided by Millennium-request copy from UA Study Coordinator) will need to be completed with as much detail as possible. Scan this document and provide any supplemental documentation available (e.g., admission summary) as a PDF document and e-mail to the following individuals at the Coordinating institution, the University of Arizona Cancer Center within 24 hours of becoming aware that the SAE has occurred. Provide a final report with the investigator's signature as soon as possible.

Study Coordinator, Lora Inclan, e-mail: linclan@azcc.arizona.edu, phone: (520) 694-9053.

IRB Coordinator, Ruth Cañamar, e-mail: rcanamar@uacc.arizona.edu, phone: (520) 626-6515.

Reporting to the UACC DSMB

The study specific SAE CRF will need to be completed with as much detail as possible. Scan this document and provide any supplemental documentation available (e.g., admission summary) as a PDF document and e-mail to the University of Arizona Cancer Center's DSMB Coordinator, Elleen Martin, e-mail: (emartin@azcc.arizona.edu), phone: (520) 626-4389 within 24 hours of becoming aware that the SAE has occurred. Provide a final report with the investigator's signature as soon as possible.

Reporting to the University of Massachusetts Memorial Cancer Center

All SAEs from either participating institution will be reported to Dr. Evens at the University of Massachusetts Memorial Cancer Center via e-mail to Andrew.Evens@umassmed.edu (within 24 hours of becoming aware that the SAE has occurred.

Reporting to the IRB

SAEs must be reported to their respective institution's IRB by the Investigator according to current policies and procedures at the institution where the study is being conducted.

Reporting to Millennium

AEs may be spontaneously reported by the patient and/or in response to an open question from study personnel or revealed by observation, physical examination, or other diagnostic procedures. Any clinically relevant deterioration in laboratory assessments or other clinical finding is considered an AE. When possible, signs and symptoms indicating a common underlying pathology should be noted as one comprehensive event. AEs which are serious must be reported to Millennium Pharmacovigilance (or designee) from the first dose of VELCADE up to and including 30 days after administration of the last dost of VELCADE Any SAE that occurs at any time after completion of VELCADE treatment or after the designated follow-up period that the sponsor-investigator and/or sub-investigator considers to be related to any study drug must be reported to Millennium Pharmacovigilance (or designee). Planned hospital admissions or surgical procedures for an illness or disease that existed before the patient was enrolled in the trial are not to be considered AEs unless the condition deteriorated in an unexpected manner during the trial (e.g., surgery was performed earlier or later than planned). All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

Since this is an investigator-initiated study, the principal investigator Daniel Persky, M.D, also referred to as the sponsor-investigator, is responsible for reporting serious adverse events (SAEs) to any regulatory agency and to the sponsor- investigator's EC or IRB. Regardless of expectedness or causality, all SAEs must also be reported to Millennium Pharmacovigilance or designee as soon as possible, but no later than 5 calendar days of the sponsor-investigator's observation or awareness of the event. See below for contact information for the reporting of SAEs to Millennium Pharmacovigilance.

The sponsor-investigator should fax the SAE Form within five calendar days after becoming aware of the event. Follow-up information on the SAE may be requested by Millennium. The

SAE report must include event term(s), serious criteria, and the sponsor-investigator's or sub-investigator's determination of both the intensity of the event(s) and the relationship of the event(s) to study drug administration. Intensity for each SAE, including any lab abnormalities, will be determined by using the NCI CTCAE version used at your institution, as a guideline, whenever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

In the event that this is a multisite study, the sponsor-investigator is responsible to ensure that the SAE reports are sent to Millennium Pharmacovigilance (or designee) from all sites participating in the study. Sub-investigators must report all SAEs to the sponsor-investigator so that the sponsor-investigator can meet his/her foregoing reporting obligations to the required regulatory agencies and to Millennium Pharmacovigilance, unless otherwise agreed between the sponsor-investigator and sub-investigator(s).

Relationship to all study drugs for each SAE will be determined by the investigator or sub-investigator by responding yes or no to the question: Is there a reasonable possibility that the AE is associated with the study drug(s)?

Sponsor-investigator must also provide Millennium Pharmacovigilance with a copy of all communications with applicable regulatory authorities related to the study or study drug(s), including, but not limited to, telephone conversation logs, as soon as possible but no later than 5 calendar days of such communication.

SAE and Pregnancy Reporting Contact Information

Millennium Pharmacovigilance
SAE and Pregnancy Reporting Contact Information:
US and Canada
24 hour helpline: 1-800-201-8725
Fax: 888-488-9697

Email for SAEs if unable to fax: wilsafety@ppdi.com

For both serious and non-serious adverse events, the investigator or sub-investigator must determine both the intensity of the event and the relationship of the event to drug administration.

Relationship to drug administration will be determined by the investigator or sub-investigator responding yes or no to the question: Is there a reasonable possibility that the adverse event is associated with the drug?

Intensity for each adverse event, including any lab abnormality, will be determined by using the NCI CTCAE, version 3.0, as a guideline, wherever possible. The criteria are available online at http://ctep.cancer.gov/reporting/ctc.html.

Additionally, the investigator must indicate the following information regarding adverse events:

- Date of onset, date of resolution
- Frequency of event (single, intermittent, continuous)
- Event outcome (resolved, ongoing, death)
- Action taken (none, medication, other)

4.3 Monitoring of Adverse Events and Period of Observation

Adverse events, both serious and non-serious, and deaths that occur during the patient's study participation will be recorded in the source documents. All SAEs should be monitored until they are resolved or are clearly determined to be due to a patient's stable or chronic condition or intercurrent illness(es).

4.3.1 Interim Analysis of Drug Toxicity

Once 20 patients have been accrued and have received at least one full cycle, the study team will review all adverse events to determine drug toxicity. This analysis will be done by the Principal Investigator and reported to the Human Subjects Protection Program at the University of Arizona in the format of a continuing review.

If unexpected, significant, or unacceptable risk to patients has been determined in the opinion of the investigator during this review, this study will be prematurely terminated. Written notification documenting the reason for study termination will be provided to Millennium and the local IRB.

In addition to our own review, the University of Arizona Cancer Center Data and Safety Monitoring Board plan for Medium risk studies will be reviewing AEs at least every six months and the local IRB is to receive SAE reports per institutional policy

4.4 Procedures for Reporting Drug Exposure During Pregnancy and Birth Events

If a woman becomes pregnant or suspects she is pregnant while participating in this study, she must inform her treating physician immediately and permanently discontinue drug therapy. Millennium must also be contacted immediately by faxing a completed Pregnancy Form to the Millennium Department of Pharmacovigilance or designee. The pregnancy must be followed through outcome (i.e. delivery, still birth, miscarriage). An SAE form will need to be completed in addition to this form.

If a female partner of a male patient becomes pregnant during the male patient's participation in this study, the sponsor-investigator must also immediately fax a completed Pregnancy Form (Appendix 8.14) to the Millennium Department of Pharmacovigilance or designee (see Section 4.2). Every effort should be made to follow the pregnancy for the final pregnancy outcome.

5 STATISTICAL PROCEDURES

5.1 Sample Size Estimation

39

5.2 Randomization and Stratification

Not applicable.

5.3 Populations for Analysis

Eligible patients must have one of the following histologies: mantle cell lymphoma, lymphoplasmacytic lymphoma (Waldenstrom's macroglobulinemia), marginal zone lymphoma, small lymphocytic lymphoma, or relapsed follicular lymphoma. They may or may not have been treated for their lymphoma.

5.4 Procedures for Handling Missing, Unused, and Spurious Data

5.5 Statistical Methods

The primary outcome measure of this Phase II trial is progression-free survival (PFS) at 2 years. This will be estimated for the cohort and presented along with appropriate confidence intervals. The Kaplan-Meier product-limit method will be used to estimate progression-free survival in the presence of censoring. A 2-year PFS of 70% or more would be considered promising, while a 2-year PFS of less than 50% would be disappointing. Thus we will test whether we can reject the null hypothesis of 50% or less versus the desired alternative rate of 70% or more. Based on an exact test for a single binomial proportion at the one-sided 0.10 significance level, a sample size of 39 patients would provide a power of 90% (i.e., maximum false negative rate of 10% and false positive rate of 10%); this calculation assumes that all patients would be followed until progression or for at least 2 years. All patients entered into the trial who receive at least one dose of treatment will be included in the analysis. With 39 patients, there will be greater than a 90% probability of observing any specific type of adverse event whose true incidence rate is 6% or higher.

5.5.1 Interim toxicity analysis

An interim analysis for toxicity will be conducted. The toxicity rate will be used as the endpoint for the interim analysis. The 60% of toxicity rate is unacceptable while 40% is acceptable. The interim analysis will be performed when the first 20 patients treated. If more than 11 toxicities are observed out of these initial 20 patients, patient enrollment will be discontinued and the study will end. Otherwise patient enrollment will continue until a total of 39 patients are enrolled.

The following Table showed the discontinuation probability when true toxicity rate is given.

True toxicity rate	*0.1*	*0.2*	*0.3*	*0.4*	*0.5*	*0.6*	*0.7*	*0.8*	*0.9*
Prob(x≥12)	*0.0000001*	*0.0001*	*0.005*	*0.0565*	*0.2517*	*0.5956*	*0.8866*	*0.9900*	*0.9999*

If the true toxicity rate is 40% and 60%, then the discontinuation probability at the interim analysis is 5.6% and 59.5%, respectively. If 12 toxicities are observed out of 20 patients, there is 80% confidence that the true toxicity rate $\geq$ 50.8% (lower bound of 1-sided 80% exact Binomial confidence interval

5.5.2 **Correlative studies**

Statistical computations will be performed using the JMP software package (SAS Institute Inc. Cary, NC). Data will be analyzed for correlation between IHC scores and histology using the Cochran-Mantel-Haenszel test (Mantel, 1963). This test is a variant of the χ^2 -test and is useful for detecting correlation between ordered rows and columns in a frequency table. This allows examination of trends to IHC scores across a progression of biological categories (MCL Vs Other Lymphomas). Further, the Cochran-Mantel-Haenszel test allows for a stratified analysis without a requirement for a large sample size within each stratum. This allows stratification of the analysis by slide, which controls for differences between slides although only 3 or 4 scores may be obtained for each slide. Because the data are not normally distributed non-parametric tests will be used. Spearman's ρ, a non-parametrical correlation statistic will be used to measure the strength of the relationship between histologic category and IHC scores.

Statistical analyses of inter-rater agreement will be carried out using the kappa statistic method (Woolson and Clark, 2002), when evaluating 2 raters simultaneously and also focuses on nominal, rather than ordinal, data. If 3 raters or institutions, as well as ordinal data are used then a more descriptive approach can be used. This can be evaluated using concordance of scoring based on (a) complete agreement and (b) agreement ± 1 numerical score (0 to 4+). Then, the percentage of scores that were in complete agreement, or in agreement within 1 numerical score, can compared among the 3 raters or 3 institutions, using data on the average result for each of the antibodies, by means of a Kruskal – Wallis test. A *P*-value < 0.05 would suggest that the 3 raters or institutions differed significantly with respect to their classifications across all antibodies. All *P*-values are 2-tailed.

6 ADMINISTRATIVE REQUIREMENTS

6.1 Good Clinical Practice

The study will be conducted in accordance with the International Conference on Harmonization (ICH) for Good Clinical Practice (GCP) and the appropriate regulatory requirement(s). The investigator will be thoroughly familiar with the appropriate use of the drug as described in the protocol and Investigator's Brochure. Essential clinical documents will be maintained to demonstrate the validity of the study and the integrity of the data collected. Master files should be established at the beginning of the study, maintained for the duration of the study and retained according to the appropriate regulations.

6.2 Ethical Considerations

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki (see section 8.5). The IRB/IEC will review all appropriate study documentation in order to safeguard the rights, safety and well-being of the patients. The study will only be conducted at sites where IRB/IEC approval has been obtained. The protocol, Investigator's Brochure, informed consent, advertisements (if applicable), written information given to the patients (including diary cards), safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the investigator. Millennium requests that informed consent documents be reviewed by Millennium or designee prior to IRB/IEC submission.

6.3 Patient Information and Informed Consent

After the study has been fully explained, written informed consent will be obtained from either the patient or his/her guardian or legal representative prior to study participation. The method of obtaining and documenting the informed consent and the contents of the consent will comply with ICH-GCP and all applicable regulatory requirement(s).

6.4 Patient Confidentiality

In order to maintain patient privacy, all data capture records, drug accountability records, study reports and communications will identify the patient by initials and the assigned patient number. The investigator will grant monitor(s) and auditor(s) from Millennium or its designees and regulatory authority(ies) access to the patient's original medical records for verification of data gathered on the data capture records and to audit the data collection process. The patient's confidentiality will be maintained and will not be made publicly available to the extent permitted by the applicable laws and regulations. All records, evaluation forms, and reports will be identified by an identification code to maintain confidentiality. A log of patients' codes, names and addresses will be kept separately. All records are to be kept in locked files.

6.5 Protocol Compliance

The investigator will conduct the study in compliance with the protocol given approval/favorable opinion by the IRB/IEC and the appropriate regulatory authority(ies). Changes to the protocol will require approval from Millennium and written IRB/IEC approval/favorable opinion prior to implementation, except when the modification is needed to eliminate an immediate hazard(s) to patients. The IRB/IEC may provide, if applicable regulatory authority(ies) permit, expedited

review and approval/favorable opinion for minor change(s) in ongoing studies that have the approval /favorable opinion of the IRB/IEC. As the coordinating center, the UACC will be responsible for preparing all protocol amendments with summary of changes document and submitting the protocol amendments to the local IRB. The UA IRB approved protocol amendment-related documents will be distributed to all participating centers by the UACC IRB Coordinator. The investigator will submit all protocol modifications to Millennium and the regulatory authority(ies) in accordance with the governing regulations. Any departures from the protocol must be fully documented in the source documents.

6.6 Registration Procedure

All ethical, regulatory, technical, and scientific approvals must be in place before study registrations will be accepted from a site. All subjects will be registered centrally with the designated Study Coordinator at the University of Arizona Cancer Center (Lora Inclan, B.A., phone: 520-694-9053; fax: 520-694-9086) prior to the start of therapy. Subjects are to be registered from 8:00 a.m. to 5:00 p.m., Mountain Standard Time, Monday through Friday, (excluding holidays). Prior to registration a fully executed consent and HIPAA, registration form and supporting documentation for reviewing eligibility criteria must be sent to the registration center. All inclusion and exclusion criteria will be confirmed with accompany source documentation to be reviewed by the Study Coordinator, Lora Inclan at the University of Arizona Cancer Center. The Study Coordinator will assign a study number.

A unique study identifier will be assigned by the University of Arizona Study Coordinator for only subjects who meet the eligibility requirements and have completed the screening visit. The unique identifier for subjects registered at the University of Arizona Cancer Center will begin with the number 001. The unique identifier will begin with the number 200 for subjects registered at the University of Massachusetts Memorial Cancer Center These numbers will be issued to subjects sequentially and no subject identification numbers will be re-assigned in the event that the subject withdraws from protocol treatment.

To register a subject, the following documents are to be completed by the research nurse or data manager and faxed (520-694-9086) or e-mailed (linclan@azcc.arizona.edu) to the Study Coordinator, Lora Inclan at the UACC:

1. Completed Registration form
2. Completed Eligibility form
3. Copy of required laboratory tests/pathology and scan reports (Label with subject's initials and obliterate image of subject's name, address, social security and medical record number)
4. Signed patient consent form
5. HIPAA authorization form

The research nurse or data manager at the participating site will then call 520-694-9053; or e-mail linclan@azcc.arizona.edu Lora Inclan, the Study Coordinator at the UACC to verify eligibility. To complete the registration process, the Coordinator at the UACC will:

1. Assign a patient study number register the subject on the study
2. Fax or e-mail the subject study number to the participating site
3. Call the research nurse or data manager at the participating site and verbally confirm registration.

Study Records – Data Collection & Recording

All study-specific data forms are to be submitted to the Study Coordinator at the University of Arizona Cancer Center at baseline (registration), at completion of VCR cycles 1-6, and at the follow-up intervals specified in section 8.1 (Appendix), including at time of relapse/progression, second malignancy, or death.

6.7 Study Data and Safety Monitoring

This research study is an investigator-sponsored trial. The University of Arizona Cancer Center will serve as the coordinating center and the study will be monitored under the auspices of the University of Arizona Cancer Center Data and Safety Monitoring Board. This is program has NCI approval and the copy is available upon request. Regulatory authorities, the IEC/IRB and/or Millennium's clinical quality assurance group may request access to all source documents, data capture records, and other study documentation for on-site audit or inspection. Direct access to these documents must be guaranteed by the investigator, who must provide support at all times for these activities.

The University of Arizona's Arizona Cancer Center (UACC) Data and Safety Monitoring Board (DSMB) will be the data safety monitoring committee of record for the conduction of this study at the University of Arizona Cancer Center and at the University of Massachusetts Memorial Cancer Center. The University of Massachusetts Memorial Cancer Center Data and Safety Monitoring Committee will also be responsible for oversight of the data generated for this trial at their institution. At the minimum, the University of Massachusetts Memorial Cancer Center will observe the Arizona Cancer Center's Protocol Data and Safety Monitoring Plan (Medium Risk) as described in the following page.

Routine study monitoring activities at the UACC (progress of study, protocol compliance and safety of subjects) will be accomplished through the UACC Quality Assurance/Quality Control Program

Routine study monitoring activities at the University of Massachusetts Memorial Cancer Center will be conducted by the University of Massachusetts Memorial Cancer Center Department staff. according to the UACC medium risk protocol data and safety monitoring plan.

The University of Arizona Cancer Center has an NCI-approved Data and Safety Monitoring Plan for clinical research. Data and systems for participating in the study will be monitored according to procedures outlined in that plan, with centralized patient screening and registration, submission and review of data, and initial verification of data as summarized below.

Protocol Data and Safety Monitoring Plan (Medium Risk)

Medium risk studies are intended to include all trials involving therapeutic intervention(s), which

are **not** designated as high risk per NCI and the IND is not held by the investigator.

Data and Safety Monitoring Plan:

1. **Identification of the DSMB obligated for oversight responsibilities:**
The University of Arizona Cancer Center Data and Safety Monitoring Board (DSMB) will provide ongoing oversight for this trial.

2. **Identification of the entity obligated for routine monitoring duties:**
Routine monitoring of the UACC will be provided by the Quality Assurance/Quality Control (QA/QC) Program to ensure that the investigation is conducted according to protocol design and regulatory requirements. Routine monitoring of U Mass data will be performed by U Mass QA/QC department.

3. **Monitoring progress and data review process:**
Routine monitoring of subject data will be conducted at least every six months.
The first routine monitoring visit will include at a minimum:
   - Informed consent – 100% of cases enrolled;
   - Subject eligibility - 50% of cases, up to two subjects;
   - Data review - 50% of cases, up to two subjects.

   All subsequent monitoring visits will consist of randomly selected subject cases based on current enrollment and include continuing review of previously selected cases, as applicable.

   A monitoring visit report and follow-up letter will be completed approximately within two weeks of the routine monitoring visit; a copy will be maintained in the study file. A query/finding form or an electronic record will also be completed by the monitor to request additional source documentation, clarification, information or corrections to the CRF and/or regulatory records. The Clinical Research Coordinator or other applicable staff responsible for the study will be given a copy of this form or will be notified of the electronic record for resolution of queries/findings. The query/finding form will be maintained with a copy of the visit report for follow-up at the next monitoring visit. Electronic records will be available in the institution database or provided by the QA/QC Program staff.

   The Principal Investigator will ensure the accuracy, completeness, legibility and timeliness of the data reported in the Case Report Form (CRF) or other acceptable formats. Source documentation supporting the study data should indicate the subject's participation in the trial and should document the dates and details of study procedures, adverse events, and patient status.

   Case report forms, which include the inclusion/exclusion criteria form, adverse event forms, serious adverse event forms and Millennium Pregnancy form should be completed via the institution database or other acceptable data formats. Trials using paper CRFs will have the data entered with a black ball-point pen or typed. Corrections to the forms should not obscure the original entry and should be made by striking the incorrect information

with a single line. Each strike should be accompanied by the initials of the corrector and the correction date. All subject forms and study files will be stored in a secure area limited to authorized staff.

Note: Routine monitoring of regulatory documents and test article will be conducted at least annually.

4. Process to implement study closure when significant risks or benefits are identified:

Please refer to Section 6.9 regarding premature closure.

5. Description of adverse events and reporting procedures:

ADVERSE EVENTS

An adverse event (AE) is any untoward medical occurrence in a patient or clinical investigation subject administered a pharmaceutical product and that does not necessarily have a casual relationship with this treatment. An AE can therefore be any unfavorable and unintended sign, symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

Any and all adverse events will be recorded on the UMC adverse events record form and reviewed by the Principal Investigator.

All adverse events will be classified using either the MedDRA term or NCI Common Terminology Criteria for Adverse Events (CTCAE) version 3.0 and will address:

- Grade
- Relationship to study drug(not related, unlikely, possible, probable, definitely)
- Causality other than study drug (disease related, concomitant medication related, intercurrent illness, other)
- Date of onset, date of resolution
- Frequency of event (single, intermittent, continuous)
- Event outcome (resolved, ongoing, death)
- Action taken (none, held, dose reduced, discontinued, medication given)

SERIOUS ADVERSE EVENTS A serious adverse event (SAE) is any untoward medical occurrence that at any dose:

1) Results in death;
2) Is life-threatening;
3) Requires in-patient hospitalization or prolongation of an existing hospital stay;
4) Results in persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions, or:
5) Is a congenital anomaly/birth defect.

Note: A SAE may also be an important medical event, in the view of the investigator that requires medical or surgical intervention to prevent one of the outcomes listed above.

All serious adverse events, regardless of attribution, and any deaths will be reported within 24 hours of notification of the event to the sponsor and DSMB Coordinator. All serious adverse events, regardless of attribution, and any deaths will be reported per institutional policy to the University of Arizona Human Subjects Protection Program.
All serious adverse events will be processed by the UACC DSMB Coordinator monthly for initial trend analysis and fully reviewed by the UACC DSMB, every six months. The UA DSMB coordinator will review the SAE reporting process to confirm reporting requirements are met.

6. **Plan for assuring data accuracy and protocol compliance:**
Routine study activity and safety information will be reported to the DSMB every six months, or more frequently if requested. These reports will include:
- Study activity, cumulative and for the period under review;
- Safety (narrative description on non-serious and serious adverse events);
- Predetermined protocol early stopping rules for efficacy/futility;
- Monitoring and protocol compliance;
- Comments;
- Attachments (AE data reviewed by the PI to compile the report, SAE letters and reports, results of any review(s), applicable correspondence with the IRB or other regulatory agencies.

Data, safety and study progress will be reported to:
- Human Subjects Protection Program (IRB) at least annually;
- Sponsor (if applicable) at least every six months.

7. **Identification of the sponsor or funding agency, as applicable:**
The PI will immediately notify; in writing, the funding agency, if applicable, any action resulting in a temporary or permanent suspension of the study.

Internal audit system.

Internal audits may be performed on this trial following the UACC DSM plan. Audits will be identified by the DSMB and conducted by an identified audit team. A QA/QC representative will coordinate the audit team functions and subject cases will be randomly selected for review. Audit result information will be provided to the PI and the DSMB for review. The following study components are reviewed, documented and reported on:
- Source documentation of subject eligibility
- Adverse events and serious adverse events
- Regulatory documentation review for IRB compliance
- Drug accountability
- Completeness and quality of data

6.8 Drug Accountability

Accountability for the drug at all study sites is the responsibility of the principal investigator. The investigator will ensure that the drug is used only in accordance with this protocol. Drug

accountability records indicating the drug's delivery date to the site (if applicable), inventory at the site (if applicable), use by each patient, and return to Millennium or disposal of the drug (if applicable and if approved by Millennium) will be maintained by the clinical site. Accountability records will include dates, quantities, lot numbers, expiration dates (if applicable), and patient numbers.

All material containing VELCADE will be treated and disposed of as hazardous waste in accordance with governing regulations.

6.9 Premature Closure of the Study

This study may be prematurely terminated, if in the opinion of the investigator or Millennium, there is sufficient reasonable cause. Written notification documenting the reason for study termination will be provided to the investigator or Millennium by the terminating party.
Circumstances that may warrant termination include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to patients
- Failure to enter patients at an acceptable rate
- Insufficient adherence to protocol requirements
- Insufficient complete and/or evaluable data
- Plans to modify, suspend or discontinue the development of the drug

Should the study be closed prematurely, all study materials must be returned to Millennium.

6.10 Record Retention

The investigator will maintain all study records according to ICH-GCP and applicable regulatory requirement(s).

6.11 Product Complaints

A product complaint is a verbal, written, or electronic expression which implies dissatisfaction regarding the identity, strength, purity, quality, or stability of a drug product. Individuals who identify a potential product complaint situation should immediately contact MedComm Solutions (see below) and report the event. Whenever possible, the associated product should be maintained in accordance with the label instructions pending further guidance from a Millennium quality representative.

A medication error is a preventable event that involves an identifiable patient and that leads to inappropriate medication use, which may result in patient harm. While overdoses and underdoses constitute medication errors, doses missed inadvertently by a patient do not. Individuals who identify a potential medication error situation should immediately contact MedComm Solutions (see below) and report the event.

For Product Complaints or Medication Errors, call MedComm Solutions at 1-866-835-2233 (US and International)

Product complaints and medication errors in and of themselves are not AEs. If a product complaint or medication error results in an SAE, an SAE form should be completed and sent to PPD (refer to Section 4.2).

7 REFERENCES

1. Adams J, Palombella VJ, Sausville EA, Johnson J, Destree A, Lazarus DD et al. Proteasome inhibitors: a novel class of potent and effective antitumor agents. Cancer Res 1999; 59 (11):2615-22.
2. Agathocleous A, Rule S, Johnson P et al. Preliminary Results of a Phase I/II Study of Weekly or Twice Weekly Bortezomib in Combination with Rituximab, in Patients with Follicular Lymphoma, Mantle Cell Lymphoma and Waldenstrom's Macroglobulinaemia. Blood 2007; 110 (11):754a (abstract 2559).
3. Airoldi I, Guglielmino R, Ghiotto F et al. Cytokine gene expression in neoplastic B cells from human mantle cell, follicular, and marginal zone lymphomas and in their postulated normal counterparts. Cancer Res 2001; 61 (4):1285-90.
4. Belch A, Kouroukis CT, Crump M et al. A phase II study of bortezomib in mantle cell lymphoma: the National Cancer Institute of Canada Clinical Trials Group trial IND.150. Ann Oncol 2007; 18 (1):116-21.
5. Blade J, Samson D, Reece D, Apperley J, Bjorkstrand B, Gahrton G et al. Criteria for evaluating disease response and progression in patients with multiple myeloma treated by high-dose therapy and haemopoietic stem cell transplantation. Myeloma Subcommittee of the EBMT. European Group for Blood and Marrow Transplant. Br J Haematol 1998; 102 (5):1115-23.
6. Camacho E, Bea S, Salaverria I et al. Analysis of Aurora-A and hMPS1 mitotic kinases in mantle cell lymphoma. Int J Cancer 2006; 118 (2):357-63.
7. Chen CI, Kouroukis CT, White D et al. Bortezomib is active in patients with untreated or relapsed Waldenstrom's macroglobulinemia: a phase II study of the National Cancer Institute of Canada Clinical Trials Group. J Clin Oncol 2007; 25 (12):1570-5.
8. Cheson BD, Horning SJ, Coiffier B et al. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol 1999; 17 (4):1244.
9. Cheson BD, Pfistner B, Juweid ME et al. Revised response criteria for malignant lymphoma. J Clin Oncol 2007; 25 (5):579-86.
10. Cusack JC, Jr., Liu R, Houston M, Abendroth K, Elliott PJ, Adams J et al. Enhanced chemosensitivity to CPT-11 with proteasome inhibitor PS-341: implications for systemic nuclear factor-kappaB inhibition. Cancer Res 2001; 61 (9):3535-40.
11. Davis TA, Grillo-Lopez AJ, White CA et al. Rituximab anti-CD20 monoclonal antibody therapy in non-Hodgkin's lymphoma: safety and efficacy of re-treatment. J Clin Oncol 2000; 18 (17):3135-43.
12. Davis TA, White CA, Grillo-Lopez AJ et al. Single-agent monoclonal antibody efficacy in bulky non-Hodgkin's lymphoma: results of a phase II trial of rituximab. J Clin Oncol 1999; 17 (6):1851-7.
13. Drach J, Kaufmann H, Pichelmayer O et al. Bortezomib, Rituximab, and Dexamethason (BORID) as Salvage Treatment in Relapsed/Refractory Mantle Cell Lymphoma: Sustained Disease Control in Patients Achieving a Complete Remission. Blood 2007; 110 (11):760a (abstract 2578).
14. Duechler M, Linke A, Cebula B et al. In vitro cytotoxic effect of proteasome inhibitor

bortezomib in combination with purine nucleoside analogues on chronic lymphocytic leukaemia cells. Eur J Haematol 2005; 74 (5):407-17.

15. Estey EH, Kurzrock R, Kantarjian HM et al. Treatment of hairy cell leukemia with 2-chlorodeoxyadenosine (2-CdA). Blood 1992; 79 (4):882-7.
16. Fisher RI, Bernstein SH, Kahl BS et al. Multicenter phase II study of bortezomib in patients with relapsed or refractory mantle cell lymphoma. J Clin Oncol 2006; 24 (30):4867-74.
17. Foran JM, Cunningham D, Coiffier B et al. Treatment of mantle-cell lymphoma with Rituximab (chimeric monoclonal anti-CD20 antibody): analysis of factors associated with response. Ann Oncol 2000; 11 Suppl 1:117-21.
18. Forstpointner R, Dreyling M, Repp R et al. The addition of rituximab to a combination of fludarabine, cyclophosphamide, mitoxantrone (FCM) significantly increases the response rate and prolongs survival as compared with FCM alone in patients with relapsed and refractory follicular and mantle cell lymphomas: results of a prospective randomized study of the German Low-Grade Lymphoma Study Group. Blood 2004; 104 (10):3064-71.
19. Fossella FV, DeVore R, Kerr RN, Crawford J, Natale RR, Dunphy F et al. Randomized phase III trial of docetaxel versus vinorelbine or ifosfamide in patients with advanced non-small-cell lung cancer previously treated with platinum-containing chemotherapy regimens. The TAX 320 Non-Small Cell Lung Cancer Study Group. J Clin Oncol 2000; 18 (12):2354-62.
20. Fossella F. Docetaxel + Cisplatin (DC) and Docetaxel + Carboplatin (DCb) vs Vinorelbine + Cisplatin (VC) in chemotherapy-naïve patients with advanced and metastatic non-small cell lung cancer (NSCLC): Results of a multicenter, randomized phase III study. European Journal of Cancer, European Cancer Conference, ECCO 11, 2001; Lisbon, Portugal. 37(suppl 6):S154. Abstract 562
21. Fridrik MA, Jager G, Kienzer HR et al. Efficacy and toxicity of 2-Chlorodeoxyadenosine (Cladribine)--2 h infusion for 5 days--as first-line treatment for advanced low grade non-Hodgkin's lymphoma. Eur J Cancer 1998; 34 (10):1560-4.
22. Ghobrial IM, Padmanabhan S, Badros A et al. Phase II Trial of Combination of Bortezomib and Rituximab in Relapsed and/or Refractory Waldenstrom Macroglobulinemia: Preliminary Results. Blood 2007; 110 (11):(abstract 4494).
23. Goy A, Bernstein S, Kahl B, Djulbegovic B, Robertson M, Vos S, et al. Durable Responses with Bortezomib in Patients with Relapsed or Refractory mantle Cell Lymphoma: Updated Time-to-Event Analyses of the Multicenter PINNACLE Study. Blood 2007;110:Abstract 125.
24. Goy A, Younes A, McLaughlin P et al. Phase II study of proteasome inhibitor bortezomib in relapsed or refractory B-cell non-Hodgkin's lymphoma. J Clin Oncol 2005; 23 (4):667-75.
25. Hideshima T, Richardson P, Chauhan D, Palombella VJ, Elliott PJ, Adams J et al. The proteasome inhibitor PS-341 inhibits growth, induces apoptosis, and overcomes drug resistance in human multiple myeloma cells. Cancer Res 2001; 61 (7):3071-6.
26. Hochster H, Weller E, Gascoyne R et al. Maintenance rituximab after CVP results in superior clinical outcome in advanced follicular lymphoma (FL): results of the E1496 phase III trial from the Eastern Cooperative Oncology Group and the Cancer and Leukemia Group B. Blood 2005; 106 (11: 349a).
27. Inwards DJ, , Fishkin PA, Hillman DW et al. Long-term results of the treatment of patients with mantle cell lymphoma with cladribine (2-CDA) alone (95-80-53) or 2-CDA and

rituximab (N0189) in the North Central Cancer Treatment Group. Cancer 2008;113(1):108-16.

28. Jagannath S, Barlogie B, Berenson J et al. A Phase 2 study of two doses of bortezomib in relapsed or refractory myeloma. Br J Haematol 2004: 127:165-172
29. Karlsson KA, Strömberg M, Jönsson V et al. Cladribine (CdA) Gives Longer Response Duration Than Fludarabine (F) and High-Dose Intermittent Chlorambucil (Chl) as First-Line Treatment of Symptomatic Chronic Lymphocytic Leukemia (CLL). First Results from the International Randomized Phase III Trial. Blood 2007 110 (11):194a (abstract 630)
30. Kay AC, Saven A, Carrera CJ et al. 2-Chlorodeoxyadenosine treatment of low-grade lymphomas. J Clin Oncol 1992; 10 (3):371-7.
31. Kononen J, Bubendorf L, Kallioniemi A et al. Tissue microarrays for high-throughput molecular profiling of tumor specimens. Nat Med 1998; 4 (7):844-7.
32. LeBlanc R, Catley LP, Hideshima T, Lentzsch S, Mitsiades CS, Mitsiades N et al. Proteasome inhibitor PS-341 inhibits human myeloma cell growth in vivo and prolongs survival in a murine model. Cancer Res 2002; 62 (17):4996-5000.
33. Lenz G, Dreyling M, Hoster E et al. Immunochemotherapy with rituximab and cyclophosphamide, doxorubicin, vincristine, and prednisone significantly improves response and time to treatment failure, but not long-term outcome in patients with previously untreated mantle cell lymphoma: results of a prospective randomized trial of the German Low Grade Lymphoma Study Group (GLSG). J Clin Oncol 2005; 23 (9):1984-92.
34. Leonard JP, Furman RR, Cheung YK et al. CHOP-R + bortezomib as initial therapy for diffuse large B-cell lymphoma (DLBCL. J Clin Oncol 2007; 25 :18S (June 20 Supplement), A-8031.
35. Lightcap ES, McCormack TA, Pien CS, Chau V, Adams J, Elliott PJ. Proteasome inhibition measurements: clinical application. Clin Chem 2000; 46 (5):673-83.
36. Maloney DG, Grillo-Lopez AJ, White CA et al. IDEC-C2B8 (Rituximab) anti-CD20 monoclonal antibody therapy in patients with relapsed low-grade non-Hodgkin's lymphoma. Blood 1997; 90 (6):2188-95.
37. Mantel N. Chi-squared tests with one degree of freedom: Extensions of the Mantel-Haenszel Procedure. J Am Stat Assoc 1963; 58:690-700.
38. Marcus R, Imrie K, Belch A et al. CVP chemotherapy plus rituximab compared with CVP as first-line treatment for advanced follicular lymphoma. Blood 2005; 105 (4):1417-23.
39. Mateos M-V, Richardson PG, Schlag R, Khuageva NK, Dimopoulos MA, Shpilberg O, et al. Bortezomib plus melphalan-prednisone continues to demonstrate a survival benefit vs melphalan-prednisone in the phase III VISTA trial in previously untreated multiple myeloma after 3 years' follow-up and extensive subsequent therapy use. Blood 2009;114:Abstract 3859.
40. McConkey DJ, Pettaway C, Elliott P, Adam J, Papandreou C, Herrmann JL et al. The proteasome as a new drug target in metastatic prostate cancer. 7th Annual Genitourinary Oncology Conference, 1999; Houston, TX. Abstract
41. McLaughlin P, Grillo-Lopez AJ, Link BK et al. Rituximab chimeric anti-CD20 monoclonal antibody therapy for relapsed indolent lymphoma: half of patients respond to a four-dose treatment program. J Clin Oncol 1998; 16 (8):2825-33.
42. Natkunam Y, Warnke RA, Montgomery K et al. Analysis of MUM1/IRF4 protein expression using tissue microarrays and immunohistochemistry. Mod Pathol 2001; 14 (7):686-94.

43. O'Connor OA. Targeting histones and proteasomes: new strategies for the treatment of lymphoma. J Clin Oncol 2005; 23 (26):6429-36.

44. O'Connor OA, Wright J, Moskowitz C et al. Phase II clinical experience with the novel proteasome inhibitor bortezomib in patients with indolent non-Hodgkin's lymphoma and mantle cell lymphoma. J Clin Oncol 2005; 23 (4):676-84.

45. Orlowski RZ, Stinchcombe TE, Mitchell BS, Shea TC, Baldwin AS, Stahl S et al. Phase I Trial of the Proteasome Inhibitor PS-341 in Patients with Refractory Hematologic Malignancies. J Clin Oncol 2002; 20 (22):4420-7.

46. Papandreou C, Daliani D, Millikan RE, Tu S, Pagliaro L, Adam J. Phase I Study of Intravenous (I.V.) Proteasome Inhibitor PS-341 in Patients (Pts) with Advanced Malignancies. Proc Am Soc Clin Oncol, 2001. Abstract 340

47. Pink MM, Pien CS, Ona VO, Worland P, Adams J, Kauffman MG. PS-341 enhances chemotherapeutic effect in human xenograft models. Proc Am Assoc Cancer Res, 2002; San Francisco, CA. Abstract 787

48. Rassidakis GZ, Jones D, Thomaides A et al. Apoptotic rate in peripheral T-cell lymphomas. A study using a tissue microarray with validation on full tissue sections. Am J Clin Pathol 2002; 118 (3):328-34.

49. Richardson PG, Barlogie B, Berenson J et al. A Phase II study of Bortezomib in Relapsed, Refractory Myeloma. N Engl J Med 2003; 348:2609-2617

50. Richardson PG, et al., Bortezomib or High-Dose Dexamethasone for Relapsed Multiple Myeloma. N Engl J Med 2005; 352:2487-98.

51. Richardson et al, Bortezomib Continues to Demonstrate Superior Efficacy Compared With High-Dose Dexamethasone in Relapsed Multiple Myeloma: Updated Results of the APEX Trial. Blood, 2005; 106: Abstract 2547.

52. Robak T, Smolewski P, Cebula B et al. Rituximab combined with cladribine or with cladribine and cyclophosphamide in heavily pretreated patients with indolent lymphoproliferative disorders and mantle cell lymphoma. Cancer 2006; 107 (7):1542-50.

53. Rosenwald A, Wright G, Wiestner A et al. The proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in mantle cell lymphoma. Cancer Cell 2003; 3 (2):185-97.

54. Rummel MJ, Chow KU, Jager E et al. Treatment of mantle-cell lymphomas with intermittent two-hour infusion of cladribine as first-line therapy or in first relapse. Ann Oncol 1999; 10 (1):115-7.

55. San Miguel JF, Schlag R, Khuageva NK, Dimopoulos MA, Shpilberg O, Kropff M, et al. Bortezomib plus melphalan and prednisone for initial treatment of multiple myeloma. N Engl J Med 2008;359(9):906-17.

56. Saven A, Burian C, Koziol JA et al. Long-term follow-up of patients with hairy cell leukemia after cladribine treatment. Blood 1998; 92 (6):1918-26.

57. Skacel M, Skilton B, Pettay JD et al. Tissue microarrays: a powerful tool for high-throughput analysis of clinical specimens: a review of the method with validation data. Appl Immunohistochem Mol Morphol 2002; 10 (1):1-6.

58. Smolewski P, Duechler M, Linke A et al. Additive cytotoxic effect of bortezomib in combination with anti-CD20 or anti-CD52 monoclonal antibodies on chronic lymphocytic

leukemia cells. Leuk Res 2006; 30 (12):1521-9.

59. Snell M, Koc O, Bahlis N et al. A phase I trial of PS-341 and fludarabine for relapsed and refractory indolent non-Hodgkin's lymphoma and chronic lymphocytic leukemia. J Clin Oncol 2006; 24 (Suppl 18):A-7580, 441s.
60. Steiner P, Neumeier H, Lightcap E, Sadis S, Pien C, Pink M et al. Adaptation of Human Tumor Cell Lines to PS-341. AACR-NCI-EORTC International Conference, 2001; Miami Beach, FL. Millennium Pharmaceuticals, Inc., 75 Sidney Street, Cambridge, MA 02139. Abstract
61. Strauss SJ, Maharaj L, Hoare S et al. Bortezomib therapy in patients with relapsed or refractory lymphoma: potential correlation of in vitro sensitivity and tumor necrosis factor alpha response with clinical activity. J Clin Oncol 2006; 24 (13):2105-12.
62. Teicher BA, Ara G, Herbst R, Palombella VJ, Adams J. The proteasome inhibitor PS-341 in cancer therapy. Clin Cancer Res 1999; 5 (9):2638-45.
63. Treon SP, Gertz MA, Dimopoulos M et al. Update on treatment recommendations from the Third International Workshop on Waldenstrom's macroglobulinemia. Blood 2006; 107 (9):3442-6.
64. VELCADE (bortezomib) [package insert]. Cambridge, MA: Millennium Pharmaceuticals, Inc.; 2003.
65. Wan WH, Fortuna MB, Furmanski P. A rapid and efficient method for testing immunohistochemical reactivity of monoclonal antibodies against multiple tissue samples simultaneously. J Immunol Methods 1987; 103 (1):121-9.
66. Woolson R, Clark W. Statistical methods for the analysis of biomedical data. In, translator and editor. New York: John Wiley and Sons; 2002; p. 263-306.
67. Yakushijin Y, Hamada M, Yasukawa M. The expression of the aurora-A gene and its significance with tumorgenesis in non-Hodgkin's lymphoma. Leuk Lymphoma 2004; 45 (9):1741-6.
68. Zinzani PL. Non-Hodgkin's lymphoma: the evolving role of purine analogues. Best Pract Res Clin Haematol 2002; 15 (3):505-16.
69. Zu Y, Steinberg SM, Campo E et al. Validation of tissue microarray immunohistochemistry staining and interpretation in diffuse large B-cell lymphoma. Leuk Lymphoma 2005; 46 (5):693-701.

8 APPENDICES

8.1 Study Flow Chart

Visits (weeks or days)	Baseline									Restaging weeks 4-8 after d1 of cycle 6	Follow-up#
	Within 8 Weeks of Registration	Within 4 Weeks of Registration	Within 2 Weeks of Registration	cycle 1	cycle 2	cycle 3	cycle 4	cycle 5	cycle 6		
Pathologic Diagnosis	X										
Tissue Microarray sample collection	X										
Bone marrow biopsy	X									X (&)	
CT chest/abdomen/pelvis		X				Xp		Xp		X	X
Study Registration (k)			X								
History/physical/PS/vital signs			X		X	X	X	X	X	X	X
CBC			X		X	X	X	X	X	X	X
Comprehensive metabolic panel (^)			X		X	X	X	X	X	X	X
Hepatitis B surface antigen(*)			X								
Serum Pregnancy test (%)			X								
LDH			X			Xp		Xp		X	X
Beta 2-microglobulin (*)			X			X		X		X	X
Serum specimen for cytokine profile			X		Xn						
For WM only: IgM, serum viscosity, SPEP with immunofixation			X			Xp		Xp		X	X
Toxicity assessment (includes FACT/GOG Neurotoxicity Questionnaire (!)					X	X	X	X	X	X	X
Optional biopsy (cycle 2, day 1)					Xn						

* Good medical practice

% Confirmation that the subject is not pregnant must be established by a negative serum β-human chorionic gonadotropin (β-hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women.

p prior to starting the cycle (within 7 days)

Every 3 months for 2 years, then annually for 5 years.

& If involved at diagnosis

n 5 milliliters of serum to be collected for cytokine profiling and 1paraffin embedded block or 10 unstained slides from the optional tumor biopsy to be performed within 2 days of Day 1of Cycle 2 ONLY

^ This includes sodium, potassium, chloride, bicarbonate, urea nitrogen, creatinine, glucose, total protein, albumin, AST, ALT, alkaline phosphatase and total bilirubin

! This includes AE assessment as well as the FACT/GOG-Neurotoxicity Questionnaire

k Within 72 hours prior to Day 1, Cycle 1

8.2 Karnofsky Performance Status Scale

The following table presents the Karnofsky performance status scale[1]:

Points	Description
100	Normal, no complaints, no evidence of disease
90	Able to carry on normal activity
80	Normal activity with effort; some signs or symptoms of disease
70	Cares for self; unable to carry on normal activity or to do active work
60	Requires occasional assistance but is able to care for most of his/her needs
50	Requires considerable assistance and frequent medical care
40	Disabled; requires special care and assistance
30	Severely disabled; hospitalization indicated. Death not imminent
20	Very sick; hospitalization necessary; active support treatment necessary
10	Moribund; fatal processes progressing rapidly
0	Dead

[1] Mor V, Laliberte L, Morris JN, Wiemann M. The Karnofsky Performance Status Scale: an examination of its reliability and validity in a research setting. *Cancer* 1984;53:2002-2007.

8.3 Body Surface Area and Creatinine Clearance Calculations

Body surface area (BSA) should be calculated using a standard nomogram that yields the following results in meters squared (m^2):

$$\text{BSA} = \sqrt{\frac{Ht(inches) \times Wt(lbs)}{3131}}$$

or

$$\text{BSA} = \sqrt{\frac{Ht(cm) \times Wt(kg)}{3600}}$$

Creatinine clearance (CrCl) can be calculated using the Cockroft-Gault equation as follows:

$$\text{CrCl (ml/min)} = \frac{\text{(140-age) (actual wt in kg)}}{\text{72 x serum creatinine (mg/dl)}}$$

For females use 85% of calculated CrCl value.

Note: In markedly obese patients, the Cockroft-Gault formula will tend to overestimate the creatinine clearance. (Adipose tissue tends to contribute little creatinine requiring renal clearance.)

8.4 New York Heart Association Classification of Cardiac Disease

The following table presents the NYHA classification of cardiac disease:

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

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

8.5 Declaration of Helsinki

World Medical Association Declaration of Helsinki:

Ethical Principles for Medical Research Involving Human Subjects

Adopted by the 18th WMA General Assembly Helsinki, Finland, June 1964 and amended by the 29th WMA General Assembly, Tokyo, Japan, October 1975 35th WMA General Assembly, Venice, Italy, October 1983 41st WMA General Assembly, Hong Kong, September 1989 48th WMA General Assembly, Somerset West, Republic of South Africa, October 1996 and the 52nd WMA General Assembly, Edinburgh, Scotland, October 2000

A. INTRODUCTION

1. The World Medical Association has developed the Declaration of Helsinki as a statement of ethical principles to provide guidance to physicians and other participants in medical research involving human subjects. Medical research involving human subjects includes research on identifiable human material or identifiable data.
2. It is the duty of the physician to promote and safeguard the health of the people. The physician's knowledge and conscience are dedicated to the fulfillment of this duty.
3. The Declaration of Geneva of the World Medical Association binds the physician with the words, "The health of my patient will be my first consideration," and the International Code of Medical Ethics declares that, "A physician shall act only in the patient's interest when providing medical care which might have the effect of weakening the physical and mental condition of the patient."
4. Medical progress is based on research which ultimately must rest in part on experimentation involving human subjects.
5. In medical research on human subjects, considerations related to the well-being of the human subject should take precedence over the interests of science and society.
6. The primary purpose of medical research involving human subjects is to improve prophylactic, diagnostic and therapeutic procedures and the understanding of the etiology and pathogenesis of disease. Even the best proven prophylactic, diagnostic, and therapeutic methods must continuously be challenged through research for their effectiveness, efficiency, accessibility and quality.
7. In current medical practice and in medical research, most prophylactic, diagnostic and therapeutic procedures involve risks and burdens.
8. Medical research is subject to ethical standards that promote respect for all human beings and protect their health and rights. Some research populations are vulnerable and need special protection. The particular needs of the economically and medically disadvantaged must be recognized. Special attention is also required for those who cannot give or refuse consent for themselves, for those who may be subject to giving consent under duress, for those who will no benefit personally from the research and for those for whom the research is combined with care.
9. Research Investigators should be aware of the ethical, legal and regulatory requirements for research on human subjects in their own countries as well as applicable international requirements. No national ethical, legal or regulatory requirement should be allowed to reduce or eliminate any of the protections for human subjects set forth in this Declaration.

B. BASIC PRINCIPLES FOR ALL MEDICAL RESEARCH

1. It is the duty of the physician in medical research to protect the life, health, privacy, and dignity of the human subject.
2. Medical research involving human subjects must conform to generally accepted scientific principles, be based on a thorough knowledge of the scientific literature, other relevant sources of information, and on adequate laboratory and, where appropriate, animal experimentation.
3. Appropriate caution must be exercised in the conduct of research which may affect the environment, and the welfare of animals used for research must be respected.
4. The design and performance of each experimental procedure involving human subjects should be clearly formulated in an experimental protocol. This protocol should be submitted for consideration, comment, guidance, and where appropriate, approval to a specially appointed ethical review committee, which must be independent of the investigator, the sponsor or any other kind of undue influence. This independent committee should be in conformity with the laws and regulations of the country in which the research experiment is performed. The committee has the right to monitor ongoing trials. The researcher has the obligation to provide monitoring information to the committee, especially any serious adverse events. The researcher should also submit to the committee, for review, information regarding funding, sponsors, institutional affiliations, other potential conflicts of interest and incentives for subjects.
5. The research protocol should always contain a statement of the ethical considerations involved and should indicate that there is compliance with the principles enunciated in this Declaration.
6. Medical research involving human subjects should be conducted only by scientifically qualified persons and under the supervision of a clinically competent medical person. The responsibility for the human subject must always rest with a medically qualified person and never rest on the subject of the research, even though the subject has given consent.
7. Every medical research project involving human subjects should be preceded by careful assessment of predictable risks and burdens in comparison with foreseeable benefits to the subject or to others. This does not preclude the participation of healthy volunteers in medical research. The design of all studies should be publicly available.
8. Physicians should abstain from engaging in research projects involving human subjects unless they are confident that the risks involved have been adequately assessed and can be satisfactorily managed. Physicians should cease any investigation if the risks are found to outweigh the potential benefits or if there is conclusive proof of positive and beneficial results.
9. Medical research involving human subjects should only be conducted if the importance of the objective outweighs the inherent risks and burdens to the subject. This is especially important when the human subjects are healthy volunteers.
10. Medical research is only justified if there is a reasonable likelihood that the populations in which the research is carried out stand to benefit from the results of the research.
11. The subjects must be volunteers and informed participants in the research project.
12. The right of research subjects to safeguard their integrity must always be respected. Every precaution should be taken to respect the privacy of the subject, the confidentiality of the patient's information and to minimize the impact of the study on the subject's physical and mental integrity

and on the personality of the subject.

13. In any research on human beings, each potential subject must be adequately informed of the aims, methods, sources of funding, any possible conflicts of interest, institutional affiliations of the researcher, the anticipated benefits and potential risks of the study and the discomfort it may entail. The subject should be informed of the right to abstain from participation in the study or to withdraw consent to participate at any time without reprisal. After ensuring that the subject has understood the information, the physician should then obtain the subject's freely-given informed consent, preferably in writing. If the consent cannot be obtained in writing, the non-written consent must be formally documented and witnessed.

14. When obtaining informed consent for the research project the physician should be particularly cautious if the subject is in a dependent relationship with the physician or may consent under duress. In that case the informed consent should be obtained by a well-informed physician who is not engaged in the investigation and who is completely independent of this relationship.

15. For a research subject who is legally incompetent, physically or mentally incapable of giving consent or is a legally incompetent minor, the investigator must obtain informed consent from the legally authorized representative in accordance with applicable law. These groups should not be included in research unless the research is necessary to promote the health of the population represented and this research cannot instead be performed on legally competent persons.

16. When a subject deemed legally incompetent, such as a minor child, is able to give assent to decisions about participation in research, the investigator must obtain that assent in addition to the consent of the legally authorized representative.

17. Research on individuals from whom it is not possible to obtain consent, including proxy or advance consent, should be done only if the physical/mental condition that prevents obtaining informed consent is a necessary characteristic of the research population. The specific reasons for involving research subjects with a condition that renders them unable to give informed consent should be stated in the experimental protocol for consideration and approval of the review committee. The protocol should state that consent to remain in the research should be obtained as soon as possible from the individual or a legally authorized surrogate.

18. Both authors and publishers have ethical obligations. In publication of the results of research, the investigators are obliged to preserve the accuracy of the results. Negative as well as positive results should be published or otherwise publicly available. Sources of funding, institutional affiliations and any possible conflicts of interest should be declared in the publication. Reports of experimentation not in accordance with the principles laid down in this Declaration should not be accepted for publication.

C. ADDITIONAL PRINCIPLES FOR MEDICAL RESEARCH COMBINED WITH MEDICAL CARE

1. The physician may combine medical research with medical care, only to the extent that the research is justified by its potential prophylactic, diagnostic or therapeutic value. When medical research is combined with medical care, additional standards apply to protect the patients who are research subjects.

2. The benefits, risks, burdens and effectiveness of a new method should be tested against those of the best current prophylactic, diagnostic, and therapeutic methods. This does not exclude the use

of placebo, or no treatment, in studies where no proven prophylactic, diagnostic or therapeutic method exists.

3. At the conclusion of the study, every patient entered into the study should be assured of access to the best proven prophylactic, diagnostic and therapeutic methods identified by the study.
4. The physician should fully inform the patient which aspects of the care are related to the research. The refusal of a patient to participate in a study must never interfere with the patient-physician relationship.
5. In the treatment of a patient, where proven prophylactic, diagnostic and therapeutic methods do not exist or have been ineffective, the physician, with informed consent from the patient, must be free to use unproven or new prophylactic, diagnostic and therapeutic measures, if in the physician's judgment it offers hope of saving life, re-establishing health or alleviating suffering. Where possible, these measures should be made the object of research, designed to evaluate their safety and efficacy. In all cases, new information should be recorded and, where appropriate, published. The other relevant guidelines of this Declaration should be followed.

8.6 FACT/GOG-Neurotoxicity Questionnaire, Version 4.0

FACT/GOG-Neurotoxicity Questionnaire, Version 4.0

By circling one (1) number per line, please indicate how true each statement has been for you during the past 7 days.

ADDITIONAL CONCERNS	Not at all	A little bit	Some-what	Quite a bit	Very much
I have numbness or tingling in my hands.....	0	1	2	3	4
I have numbness or tingling in my feet.....	0	1	2	3	4
I feel discomfort in my hands.....	0	1	2	3	4
I feel discomfort in my feet.....	0	1	2	3	4
I have joint pain or muscle cramps.....	0	1	2	3	4
I feel weak all over.....	0	1	2	3	4
I have trouble hearing.....	0	1	2	3	4
I get a ringing or buzzing in my ears.....	0	1	2	3	4
I have trouble buttoning buttons.....	0	1	2	3	4
I have trouble feeling the shape of small objects when they are in my hand.....	0	1	2	3	4
I have trouble walking.....	0	1	2	3	4

Sources: Cella DF, Tulsky DS, Gray G, Sarafian B, Lloyd S, Linn E, et al. The functional assessment of cancer therapy (FACT) scale: development and validation of the general measure. *J Clin Oncol* 1993;11(3):570-79.

8.7 Ann Arbor Classification (AJCC Manual for Staging of Cancer, 6th ed., 2002)

STAGE II Involvement of two or more lymph node regions on the same side of the diaphragm (II) or localized involvement of an extralymphatic organ or site and its associated regional lymph nodes (IIE).

STAGE III Involvement of lymph node regions on both sides of the diaphragm (III), which may be accompanied by localized involvement of an associated extralymphatic organ or site (IIIE) or spleen (IIIS) or both (IIISE).

STAGE IV Diffuse or disseminated involvement of one or more extra lymphatic organs with or without associated lymph node involvement, or isolated extralymphatic organ involvement with distant (non-regional) nodal involvement.

A = Asymptomatic

B = Unexplained fever (38°C), night sweats, unexplained weight loss > 10% of body weight over the previous six months

"Bulky" = mediastinal mass > 1/3 of the maximum chest diameter (i.e., internal dimension of the thoracic cavity measured at its widest point per radiograph) or any other mass ≥ 10 cm in maximum diameter.

8.8 International Working Group Response Criteria (Cheson et al, 1999)

Response Category	Physical Examination	Lymph Nodes	Lymph Node Masses	Bone Marrow
CR (complete response)	Normal	Normal	Normal	Normal
CRu (complete response, unconfirmed)	Normal	Normal	Normal	Indeterminate
	Normal	Normal	>75% decrease	Normal or indeterminate
PR (partial response)	Normal	Normal	Normal	Positive
	Normal	≥50% decrease	≥50% decrease	Irrelevant
	Decrease in liver/spleen	≥50% decrease	≥50% decrease	Irrelevant
Progression/relapse	Enlarging liver/spleen, new sites	New or increased	New or increased	Reappearance

If PET scan is also used for staging, revised Response Criteria should be used (Cheson et al, 2007)

8.9 Updated Response Criteria from the 3rd International Workshop on Waldernstrom's Macroglobulinemia (Treon et al, 2006)

Response Category	Criteria
CR (Complete Response)	Disappearance of monoclonal protein by immunofixation; no histologic evidence of bone marrow involvement, resolution of any adenopathy/organomegaly (confirmed by CT scan), or signs or symptoms attributable to WM. Reconfirmation of the CR status is required at least 6 weeks apart with a second immunofixation.
PR (Partial Response)	At least 50% reduction of serum monoclonal IgM concentration on protein electrophoresis and at least 50% decrease in adenopathy/organomegaly on physical examination or on CT scan. No new symptoms or signs of active disease.
MR (Minor Response)	At least 25% but less than 50% reduction of serum monoclonal IgM by protein electrophoresis. No new symptoms or signs of active disease.
SD (Stable Disease)	A less-than25% reduction and less-than-25% increase of serum monoclonal IgM by electrophoresis without progression of adenopathy/organomegaly, cytopenias, or clinically significant symptoms due to disease and/or signs of WM.
PD (Progressive Disease)	At least 25% increase in serum monoclonal IgM by protein electrophoresis confirmed by a second measurement or progression of clinically significant findings due to disease (i.e., anemia, thrombocytopenia, leucopenia, bulky adenopathy/organomegaly) or symptoms (unexplained recurrent fever of at least 38.4°C, drenching night sweats, at least 10% body weight loss, or hyperviscosity, neuropathy, symptomatic cryoglobulinemia, or amyloidosis) attributable to WM.

8.10 Protocol Signature Page

Protocol Signature Page

Protocol Title:

"A Phase II, Open-Label Study of Bortezomib (Velcade®), Cladribine, and Rituximab (VCR) in Advanced, Newly Diagnosed and Relapsed/Refractory Mantle Cell and Indolent Lymphomas"

Protocol Version/Date:

August 15, 2017

I agree to conduct the study as outlined in the protocol and to comply with all the terms and conditions set out therein. I confirm that I will conduct the study in accordance with FDA and ICH GCP guidelines and all other applicable regulatory requirements. I also will ensure that sub-investigator(s) and other relevant members of my staff have access to copies of this protocol.

Signature of Principal Investigator	Date of Signature

Daniel Persky, MD

Printed Name of Principal Investigator

University of Arizona Cancer Center

Institution Name

1515 N Campbell Ave, Tucson, AZ 85724

Institution address

APPENDIX 8.11 TISSUE SUBMISSION FORM

A Phase II, Open-Label Study of Bortezomib (Velcade®), Cladribine, and Rituximab (VCR) in Advanced, Newly Diagnosed and Relapsed/Refractory Mantle Cell and Indolent Lymphomas

Subject Initials: ☐☐☐ Subject study ID: ☐☐☐

Date of Submission to Rimsza laboratory (MM/DD/YYYY): ☐☐/☐☐/☐☐☐☐

Type of sample and timepoint collected (MM/DD/YYYY and 24 hour clock format)

Tumor block pre-study: # blocks:☐ Date: ☐☐/☐☐/☐☐☐☐ Time: ☐☐:☐☐

Tumor unstained slides pre-study: # ☐☐ Date: ☐☐/☐☐/☐☐☐☐ Time: ☐☐:☐☐

Tumor block C2D1: # blocks:☐☐ Date: ☐☐/☐☐/☐☐☐☐ Time: ☐☐:☐☐

Tumor unstained slides C2D1: # ☐☐ Date: ☐☐/☐☐/☐☐☐☐ Time: ☐☐:☐☐

Blood, serum, pre study: # 1 ml aliquots:☐Date: ☐☐/☐☐/☐☐☐☐ Time: ☐☐:☐☐

Blood, serum, C2D1: # 1 ml aliquots:☐ Date: ☐☐/☐☐/☐☐☐☐ Time: ☐☐:☐☐

*Blood, fresh pre study: # tubes:☐ Date: ☐☐/☐☐/☐☐☐☐ Time: ☐☐:☐☐

*Blood, fresh C2D1: # tubes:☐ Date: ☐☐/☐☐/☐☐☐☐ Time: ☐☐:☐☐

* for University of Arizona site only

*** write in an "X" in the box if blocks need to be returned to the sender: ☐

Central Laboratory Use Only

Central Laboratory ID #: ☐☐☐ Usable as received ☐

Date specimen (s) received: ☐☐/☐☐/☐☐☐☐ Not usable as received ☐

Time specimen received: ☐☐:☐☐ Not usable as received Inadequate submission ☐

Comments: _____

APPENDIX 8.12 SUBJECT REGISTRATION FORM

A Phase II, Open-Label Study of Bortezomib (Velcade®), Cladribine, and Rituximab (VCR) in Advanced, Newly Diagnosed and Relapsed/Refractory Mantle Cell and Indolent Lymphomas

Subject Registration Date: _(MM/DD/YYYY): ☐☐/☐☐/☐☐☐☐

Expected Start Date of Treatment: __(MM/DD/YYYY): ☐☐/☐☐/☐☐☐☐

Subject Initials: ☐☐☐ **Subject study ID**: ☐☐☐

***** DOES THIS PATIENT MEET ALL ELIGIBILITY CRITERIA LISTED IN THE PROTOCOL?** __ Yes __ No

Physician: _____ **Site:** _____

Registrant: _____ **Phone:** _____

CONFIRMATION OF ENROLLMENT

Subject Initials: ☐☐☐ **Subject study ID**: ☐☐☐

APPENDIX 8.13 SUBJECT ELIGIBILITY FORM (Page 1)

A Phase II, Open-Label Study of Bortezomib (Velcade®), Cladribine, and Rituximab (VCR) in Advanced, Newly Diagnosed and Relapsed/Refractory Mantle Cell and Indolent Lymphomas"

Subject Initials: ☐☐☐ **Subject study ID**: ☐☐☐

Inclusion Criteria

1. Voluntary written informed consent before performance of any study-related procedure not part of normal medical care, with the understanding that consent may be withdrawn by the subject at any time without prejudice to future medical care. ☐
2. Female subject is either post-menopausal or surgically sterilized or willing to use an acceptable method of birth control (i.e., a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) for the duration of the study. ☐
3. Male subject agrees to use an acceptable method for contraception for the duration of the study. ☐
4. Biopsy-proven mantle cell, marginal zone, lymphoplasmacytic, small lymphocytic lymphoma, or follicular lymphoma ☐
5. CD20-positive disease ☐
6. For patients with marginal zone, lymphoplasmacytic, small lymphocytic, or follicular lymphoma – at least one criterion for initiation of treatment must be met:
    a. Symptomatic disease ☐
    b. Cytopenia related to lymphoma ☐
    c. Leukemic phase (> 5,000 malignant lymphocytes/µl) ☐
    d. Mass over 5 cm in greatest diameter ☐
    e. For lymphoplasmacytic lymphoma: additional treatment criteria are serum viscosity ≥ 4 cp, serum monoclonal protein > 5 g/L, concurrent primary systemic AL amyloidosis, cold agglutinin disease ☐
7. Age over 18 ☐
8. Prior treatment with bortezomib and/or rituximab is acceptable ☐
9. For follicular lymphoma only, at least one prior treatment ☐

APPENDIX 8.13 SUBJECT ELIGIBILITY FORM (Page 2)

Subject Initials: ☐☐☐ **Subject study ID**: ☐☐☐

Exclusion Criteria

- Patient has a platelet count of $< 100 \times 10^9$/L within 14 days before enrollment, unless due to bone marrow infiltration with lymphoma, or due to autoimmune thrombocytopenia because of lymphoma. ☐
- Patient has an absolute neutrophil count of $< 1.0 \times 10^9$/L within 14 days before registration, unless due to bone marrow infiltration with lymphoma. ☐
- Patient has a calculated or measured creatinine clearance of <20 mL/minute within 14 days before registration. (Creatinine Clearance is indicated through the Serum Creatinine. If the Serum Creatinine is abnormal, the physician may then due a 24 hour urine to further clarify Creatinine Clearance. A 24 hour urine test is not required per study.) ☐
- Patient has ≥ Grade 2 peripheral neuropathy within 14 days before registration. ☐
- Myocardial infarction within 6 months prior to registration or has New York Heart Association (NYHA) Class III or IV heart failure (see section 8.4), uncontrolled angina, severe uncontrolled ventricular arrhythmias, or electrocardiographic evidence of acute ischemia or active conduction system abnormalities. Prior to study entry, any ECG abnormality at Screening has to be documented by the investigator as not medically relevant. ☐
- Patient has hypersensitivity to bortezomib, boron or mannitol. ☐
- Female subject is pregnant or breast-feeding. Confirmation that the subject is not pregnant must be established by a negative serum β-human chorionic gonadotropin (β-hCG) pregnancy test result obtained during screening. Pregnancy testing is not required for post-menopausal or surgically sterilized women. ☐
- Patient has received other investigational drugs with 14 days before registration. ☐
- Serious medical or psychiatric illness likely to interfere with participation in this clinical study. ☐
- Diagnosed or treated for another malignancy within 3 years of registration, with the exception of complete resection of basal cell carcinoma or squamous cell carcinoma of the skin, an in situ malignancy, or low-risk prostate cancer after curative therapy. ☐
- CNS involvement with lymphoma. ☐
- Known HIV-positive. ☐
- History of disease refractory to a purine analog (defined as remission duration of < 6 months to therapy that included fludarabine, pentostatin, or cladribine). ☐
- History of intolerance of bortezomib, cladribine, or rituximab. ☐

Appendix 8.14: Pregnancy Reporting Form

MILLENNIUM
THE TAKEDA ONCOLOGY COMPANY

Pregnancy Form v03Nov2008 (IIS)

Report Type: ❒ Initial ❒ Follow-up	Date of Report: ___/___/_____ DD MM Yr

REPORTER INFORMATION: (Please forward if an alternative physician is more appropriate)		
Reporter name: _____	Title: _____	
Address: _____	Telephone No.: _____	Fax No. _____
City, State/Province: _____	Postal Code: _____	Country: _____

FATHER'S INFORMATION ❒ Father Unknown	
Initials: _____ Date of Birth: ___/___/_____ (DD MM Yr) or Age: _____years	
Participating in an MPI clinical study? ❒ No ❒ Yes *If no*, what company product was taken: _____ *If yes, please provide:* Study drug: _____ Protocol No: _____ Center No: _____ Patient No: _____	
Medical / Familial / Social History (i.e. Include chronic illnesses: specify, familial birth defects/genetic/chromosomal disorders; habitual exposure: specify, alcohol/tobacco; drug exposure: specify, substance abuse and medication use. Please include drug treatment prior to or around the time of conception and/or during pregnancy) _____ _____	Race: _____ Occupation: _____ Number of children: _____

MOTHER'S INFORMATION:

Initials: _____ Date of Birth: ___/___/_____ or Age: _____years
DD MM Yr

Participating in an MPI clinical study? ❒ No ❒ Yes
If no, what company product was taken: _____
If yes, please provide: Study drug: _____ Protocol No: _____
Center No: _____ Patient No: _____

Race: _____
Occupation: _____

Medical / Familial / Social History
(i.e. Include alcohol/tobacco and substance abuse; complications of past pregnancy, labor/delivery, fetus/baby; illnesses during this pregnancy; assisted conception: specify; other disorders including familial birth defects/genetic/chromosomal disorders; method of diagnosis consanguinity, etc.)

_____

_____

_____

Number of previous pregnancies: Full term ____ Pre-term _____

Outcomes of previous pregnancies:
(Please indicate number of occurrences)

- Spontaneous abortion: _____
- Therapeutic abortion: _____
- Elective abortion: _____
- Other: _____
- Normal live birth: _____
- Children born with defects: _____
- Stillbirth: _____
- Outcome unknown: _____

MOTHER'S DRUG EXPOSURE INFORMATION

Please include medical prescriptions, vaccinations, medical devices, OTC products, pregnancy supplements (such as folic acid, multivitamins)

Product Name	Dosage	Route administered to patient	Date of first use (DD/MM/Yr)	Date of end treatment (DD/MM/Yr)	Indication	Contraindicated to pregnancy
			(__/__/__)	(__/__/__)		❒Yes ❒No ❒Unk
			(__/__/__)	(__/__/__)		❒Yes ❒No ❒Unk
			(__/__/__)	(__/__/__)		❒Yes ❒No ❒Unk
			(__/__/__)	(__/__/__)		❒Yes ❒No ❒Unk

CURRENT PREGNANCY INFORMATION

Period at exposure: _____ weeks Trimester (1) (2) (3)
Date of last menstrual period: ____/____/_____ ❒ Unknown
DD MM Yr

Pregnancy Status

❒ Pregnancy Ongoing
Estimated date of delivery: ___/___/_____
DD MM Yr
❒ Live Birth
❒ Stillbirth
❒ Early Termination
- ❒ Spontaneous abortion*
- ❒ Therapeutic abortion*
- ❒ Elective abortion*
- ❒ Other*:_____

If box is checked, please note reason in "Additional Details" section below

Fetal/Neonatal Status

❒ Normal
❒ Birth defect (structural/chromosomal disorder)*
❒ Other (non-structural, premature birth, intrauterine death/stillbirth)*

If box is checked, please note details in "Additional details" section below

Additional Details:

Is there evidence of a defect from a prenatal test? ❒Yes ❒No

If yes, indicate which test(s) showed evidence of birth defect:

❒Ultrasound ❒Amniocentesis ❒Maternal Serum-Alpha-Fetoprotein
❒Chorionic Villi Sampling ❒Human Chorionic Gonadotropin ❒Other:_____

Please specify details of defect(s), disorder(s), and/or other anomaly(ies): _____

_____

What are the defect(s) attributed to:_____

Infant Information:

Gestational weeks at birth or at termination: _____weeks

Date of birth or termination: ____/____/_____
DD MM Yr

If multiple births (e.g. twins), indicate number: _____
(Please complete separate form for each child)

Birth Order (1, 2, 3, etc.) _____

Breast-fed: ❒Yes ❒ No ❒ Unk

Method of delivery: ❒ Normal vaginal ❒ Caesarean section
❒ Other: _____

Sex: ❒ Male ❒ Female ❒ Unk

Length: _____ ❒ cm ❒ in

Weight: _____ ❒ g ❒ lbs

Head circumference: _____ ❒ cm ❒ in

Apgar score (0-10) at 1 minute: ____ ❒ Unk

Apgar score (0-10) at 5 minute: ____ ❒ Unk

Resuscitation required: ❒ Yes ❒ No ❒ Unk

Admission to intensive care required:
❒ Yes ❒ No ❒ Unk

Additional Notes:

_____

_____

_____

Please attach RELEVANT LABORATORY TESTS AND PROCEDURES (e.g. results of ultrasounds, amniocentesis, chorionic villi sampling, or miscellaneous testing as applicable). In the case of an abnormal evolution or outcome, please send copies of results of all relevant laboratory testing and procedures, including pathology results of products of conception and or autopsy reports if applicable. Please submit any additional relevant information on a separate sheet.

Investigator signature: _____ Date: ____/____/_____
DD MM Yr

Investigator Name: _____