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Randomized Phase II Study of Dose-Adjusted EPOCH-Rituximab-Bortezomib Induction Followed by Bortezomib Maintenance versus Observation in Untreated Mantle Cell Lymphoma with Microarray Profiling and Proteomics

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Investigational Agents: None

Commercial Agents: Etoposide, Prednisone, Vincristine, Cyclophosphamide, Doxorubicin, Rituximab, Bortezomib, Filgrastim

PRÉCIS

Background:

- Mantle cell lymphoma (MCL) presents a clinical challenge because it is aggressive and incurable with chemotherapy. Therefore, novel treatment approaches are needed.
- MCL has overexpression of NF-kappa B (NF-κB), a transcription factor that affects cell growth and survival, and cyclin D1 that affects cell cycle and growth. These proteins appear to be involved in the pathogenesis of MCL.
- Bortezomib, a proteasome inhibitor that inhibits NF-κB and cyclin D1, has demonstrated activity in patients with relapsed or refractory MCL.
- Dose-adjusted-EPOCH-R has excellent activity in MCL, with a complete response (CR) rate of 92%, but patients eventually relapse.

Objective:

- Determine the PFS and OS of DA-EPOCH-RB followed by bortezomib maintenance versus observation

Eligibility:

- Diagnosis of mantle cell lymphoma
- No prior treatment except for local radiation or a short course of steroids for control of symptoms,
- Age $\geq$ 18 years old
- Adequate major organ function unless impairment is due to lymphoma.

Study Design:

- To assess the clinical activity and biological effects of bortezomib, patients will initially receive one cycle of bortezomib alone with sequential tumor biopsies for microarray analysis.
- All patients will then receive Dose-adjusted (DA)-EPOCH-RB for 6 cycles, and if they have at least a PR, this will be followed by randomization to either immediate bortezomib maintenance x 18 months, or to observation, followed by bortezomib if progression occurs. This study has as a primary goal, to describe progression free survival (PFS) and overall survival of early bortezomib maintenance versus observation following induction with bortezomib followed by DA-EPOCH-RB. Important secondary goals are to assess response and toxicity to bortezomib alone or DA-EPOCH-RB, to evaluate time to progression after receiving bortezomib following progression on an observation arm, and to assess the biological effects of bortezomib on untreated MCL.

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STATEMENT OF COMPLIANCE

The trial will be carried out in accordance with International Conference on Harmonisation Good Clinical Practice (ICH GCP) and the following:

- United States (US) Code of Federal Regulations (CFR) applicable to clinical studies (45 CFR Part 46, 21 CFR Part 50, 21 CFR Part 56, 21 CFR Part 312, and/or 21 CFR Part 812)

National Institutes of Health (NIH)-funded investigators and clinical trial site staff who are responsible for the conduct, management, or oversight of NIH-funded clinical trials have completed Human Subjects Protection and ICH GCP Training.

The protocol, informed consent form(s), recruitment materials, and all participant materials will be submitted to the Institutional Review Board (IRB) for review and approval. Approval of both the protocol and the consent form must be obtained before any participant is enrolled. Any amendment to the protocol will require review and approval by the IRB before the changes are implemented to the study. In addition, all changes to the consent form will be IRB-approved; an IRB determination will be made regarding whether a new consent needs to be obtained from participants who provided consent, using a previously approved consent form.

1 INTRODUCTION

1.1 STUDY OBJECTIVES

1.1.1 Primary

- Determine the PFS and OS of DA-EPOCH-RB followed by bortezomib maintenance versus observation

1.1.2 Secondary

- Determine response to bortezomib pre-DA-EPOCH-RB in "window of opportunity"
- Determine response and toxicity of DA-EPOCH-RB
- Determine response and PFS of bortezomib at disease progression in the observation arm
- Compare time to non-protocol treatment in the maintenance versus observation arms
- Assess effects of bortezomib on mantle cell lymphoma by microarray, proteomics and genomic methylation microarrays
- Correlate microarray, genomic methylation and proteomic findings with clinical outcomes

1.1.3 Exploratory

- Explore molecular and genomic studies in tissue that may predict response and outcomes

1.2 BACKGROUND AND RATIONALE

Hypothesis: Mantle cell lymphoma (MCL), an aggressive B-cell neoplasm, has only recently been recognized as a distinct biologic entity and compromises approximately 2.5 to 4% of all NHL in the U.S. It occurs more commonly in the elderly, has a male predominance and typically presents at an advanced stage. The clinical behavior of MCL tends to be aggressive but can vary from relatively indolent to very aggressive. Although the disease responds to chemotherapy, it is

usually incurable and responses are of short duration with a median survival of 3 – 4 years. [1] Studies looking at various chemotherapy regimens in untreated mantle cell lymphoma have demonstrated event-free survivals ranging from 8 to 19 months and disease-free survivals ranging from 19 to 25 months, depending on the series (Table 1).

Series	n	PR (%)	CR (%)	Median DFS (months)	Median PFS (months)	Median Survival (months)
G.A. Velder[1,2]	41	---	32	25	---	24
W.Hiddenmann[3]	45	52	17	---	8	28
H. Samaha[4]	121	28	68	---	---	38
I. Teodorovic[5]	65	---	52	20	19	45
E. Zucca[6]	26	---	50	19	---	33
J. Armitage[7]	83	---	---	---	---	32

The use of autologous stem cell transplantation and high dose therapy in the treatment of newly diagnosed mantle cell lymphoma is controversial. Khouri et al tested an aggressive regimen of hyper-CVAD and high-dose methotrexate/cytarabine followed by stem cell transplantation in patients with relapsed or previously untreated mantle cell lymphoma.[8] Among the 25 previously untreated patients, the overall survival (OS) and event-free survival (EFS) at 3 years were 92% and 72% respectively. In contrast, the EFS at 3 years was 17% in the previously treated patients. Although the results are impressive in the untreated group, the results from the previously treated group suggest that this combination approach can salvage few if any patients and is therefore unlikely to be curative in the untreated group. A recent study published by Mangel et al. prospectively evaluated intensive chemotherapy and high-dose chemotherapy plus rituximab for newly diagnosed advanced stage mantle cell lymphoma. Of 20 patients treated, 17 remain in remission at a median of 30 months from diagnosis.[9] However, longer follow-up is required to assess the impact of this strategy on PFS and OS. Other studies have shown little evidence that high-dose treatment with transplant is potentially curative. Freedman et al treated 28 MCL patients with high-dose chemo-radiotherapy and anti-B-cell monoclonal antibody purged autologous bone marrow transplantation.[10] Twenty patients had received prior regimens before transplant, and 8 were in first CR/PR following CHOP. Nineteen (68%) patients relapsed at a median of 21 months, and of 8 patients in first CR/PR, 5 had relapsed. With a median follow-up of 24 months, DFS and OS were estimated to be 31% and 62%, respectively, at 4 years, indicating that high dose chemotherapy with autologous stem-cell support cures few if any patients.

Thus, with anthracycline based chemotherapy or high dose chemotherapy, MCL is largely incurable and therefore new treatment approaches are needed. Dose-adjusted EPOCH in combination with rituximab has good activity in mantle cell lymphoma with a CR rate of 92%. However, most patients eventually have disease relapse and thus additional approaches to treatment of this disease are worth investigating with the hope of improving time to treatment failure and curability rates.

MCL is characterized by t (11; 14) (q13; q32) resulting in over-expression of cyclin D1 which is involved in regulation of the cell cycle - it controls G1 progression and G1 – S transition. NF-κB, a transcription factor involved in immune and inflammatory cellular responses affecting both cell growth and survival, appears to have an important role in the pathogenesis of aggressive lymphoid malignancies including MCL.[11] Therefore, therapeutic strategies that target NF-κB, such as proteasome inhibition by Bortezomib, possibly in combination with conventional chemotherapy, are worthwhile investigating.

In eukaryotic cells, the ubiquitin proteasome pathway plays an essential role in the degradation of most intracellular proteins. The 26 S proteasome degrades regulatory proteins involved in cell cycle control. Some targets of this degradation are p53, p21, NF-κB, IκB and bcl-2. The NF-κB/Rel transcription factors integrate diverse intracellular signaling pathways that are activated during normal cellular differentiation and during immune responses. [12-15] NF-κB dependent transcriptional activity is mediated by dimers of NF-κB family members (p50/105, p52/100, p65/RelA, RelB, or c-Rel), and is regulated by members of the IκB family of inhibitors, principally IκBα, which binds to NF-κB dimers and retains them in the cytoplasm. Upon phosphorylation by the IKK complex, IκBα is targeted for ubiquitination and proteasomal degradation, and released NF-κB dimers can translocate to the nucleus and activate transcription of target genes.[13] NF-κB target genes encode diverse mediators of immune responses as well as regulators of cellular proliferation and apoptosis. The expression of these target genes varies, in part, with the cell type in which NF-κB is activated. NF-κB activity is also critical for normal B cell development and survival.[16] NF-κB activation by mitogenic stimuli is normally self-limited, but constitutive nuclear NF-κB has been found in several types of cancers, raising the possibility that NF-κB may contribute to malignant transformation or progression[11,17]. For example, in cell lines and Hodgkin's disease, mutations of IκBα gene result in its functional inactivation and the accumulation of p50/RelA heterodimers in the nucleus.[17-21] In other types of lymphoid malignancies, constitutive NF-κB activity can occur occasionally due to translocations involving the NF-κB2 gene that disrupt its carboxy terminus,[22,23] or by amplification of the *c-rel* locus.[24,25] Of interest is evidence that NF-κB transcriptionally activates bcl-2.

The ability of NF-κB to inhibit responses to cancer therapeutic agents may contribute to the poor outcome of MCL, and inhibition of NF-κB can synergize with chemotherapy to kill tumor cells.[11] P21 and p27, two cdk inhibitory proteins and members of the INK-4 family are important for cell cycle regulation.[26] MCL cells often have low levels of p21 and p27, and low p27 levels have been associated with a poor prognosis.[27] Both p21 and p27 are up regulated in MCL cells after Bortezomib treatment.[28] Two recently published studies [28,29] have demonstrated that inhibition of the proteasome induces cell cycle arrest and apoptosis in MCL lines. In the first of these,[28] it is demonstrated that inactivating NF-κB in MCL lines with the proteasome inhibitor Bortezomib or the PIκBα inhibitor Bay 11, blocks MCL cell growth leading to tumor cell death. The mechanism of blocking cell growth was via cell cycle arrest and induction of cell death, both of which involve inhibition of constitutive NF-κB activation. This cell cycle arrest was associated with inhibition of cyclin D1 expression. A second study [11] looked at proteasome inhibition in MCL lines that closely resembled blastic MCL with deletion of p15 and p16 (Granta 519) and mutation of P53 (NCEB)). In this study, cell cycle arrest and apoptosis were accompanied by accumulation of the cdk inhibitor p21 in both cell lines. These results suggest

that inhibition of NF-κB, through proteasome inhibition by Bortezomib, may induce tumor cell apoptosis or decrease bcl-2 associated drug resistance.

Two studies, recently presented in abstract form, have demonstrated good activity of Bortezomib in the setting of relapsed and refractory mantle cell lymphoma. In the first of these,[30] the dose of Bortezomib was 1.5mg/m^2 IV on days 1, 4, 8 and 11 every 21 days. Of 15 evaluable patients, 8/15 patients responded with 3 CRs and 5 PRs (RR 53%). In the second study,[31] using a dose of 1.3mg/m^2 IV twice weekly for 2 out of every 3 weeks, there was a PR rate of 38.5% (5 of 13 evaluable patients).

1.2.1 Bortezomib 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. Bortezomib is a dipeptidyl boronic acid derived from leucine and phenylalanine, which is a potent and reversible inhibitor of the proteasome. The ubiquitin-proteasome pathway is essential for the degradation of most short- and long-lived intracellular proteins in eukaryotic cells. The 26S proteasome, universally present and abundant in all eukaryotic cells, is an ATP-dependent multicatalytic protease that is central to the degradative pathway. The 26S proteasome functions not only in a housekeeping role to eliminate damaged or misfolded proteins but also as a critical regulator of multiple cellular processes by virtue of the many regulatory proteins governing the cell cycle, transcription factor activation, apoptosis, and cell trafficking that are substrates for proteasome-mediated degradation. The proteasome is the final degradative enzyme involved in an important catabolic pathway for many intracellular regulatory proteins including NF-κB, p53, and the cyclin-dependent kinase inhibitors p21 and p27. Notably, Bortezomib induces apoptosis in cells that over express bcl-2, a genetic trait that confers unregulated growth and resistance to conventional chemotherapeutics. Targeting the proteasome has emerged as a novel approach to cancer therapy. Bortezomib is currently approved by the United States Food and Drug Administration (US FDA) and it is registered in Europe for the treatment of multiple myeloma patients who have received at least one prior therapy and have demonstrated disease progression on the last therapy.

1.2.2 Bortezomib 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 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 Investigator's Brochure

1.2.3 Clinical Pharmacokinetics and Pharmacodynamics of Bortezomib

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. In solid tumor subjects, the mean terminal elimination half-life of bortezomib was 9.06 hours. The mean area under the curve $(AUC)_{(0\text{-}24)}$ after the first dose (1.3 mg/m^2) of bortezomib was 48.2 hr*ng/mL. The average clearance of bortezomib following a single 1.3 mg/m^2 dose was 49.0 L/hr. However, the AUC increased to 81.0 hr*ng/mL after the third dose in the first cycle as a result of a reduction in systemic clearance to 28.2 L/hr with a consequent increase in elimination half-life to 54.0 hours. Clinical experience has shown that the change in clearance does not result in overt toxicity from accumulation in this multidose regimen in humans. The overall disposition of bortezomib is consistent with a 2-compartment PK model, although the existence of a third compartment cannot be excluded at this time due to the lack of supportive steady-state PK data 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.

1.2.4 Clinical Experience with Bortezomib

It is estimated that as of June 2005, more than 24,000 patients have been treated with bortezomib, including patients treated through Millennium-sponsored clinical trials, Investigator-Initiated Studies, the US NCI Cancer Therapy Evaluation Program (CTEP), and with commercially available drug. Bortezomib 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 bortezomib 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 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 bortezomib 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 bortezomib 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 bortezomib in subjects with multiple myeloma were investigated in two phase 2 clinical studies, studies M34100-024 (subjects with first relapse) and M34100-025 (subjects with second or greater relapse and refractory to their last prior therapy). In M34100-025, 202 heavily pre-treated subjects with refractory multiple myeloma after at least 2 previous treatments received bortezomib 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), also referred to as the APEX study, was designed to determine whether bortezomib 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 bortezomib relative to high-dose dexamethasone, and whether treatment with bortezomib 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 (bortezomib: 331; dexamethasone: 332). Patients randomized to bortezomib 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 bortezomib 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 bortezomib arm. Median TTP was 6.2 months for the bortezomib arm and 3.5 months for the dexamethasone arm (P<.0001). CR (complete response) + PR (partial response) was 38% with bortezomib vs. 18% with dexamethasone (P<.0001). CR was 6% with bortezomib vs. <1% with dexamethasone (P<.0001). The CR + nCR rate was13% with bortezomib vs. 2% with dexamethasone. In patients who had received only one prior line of treatment (bortezomib: 132; dexamethasone: 119), CR + PR was 45% with bortezomib 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 bortezomib arm vs. patients on the dexamethasone arm. The probability of survival at one year was 80% for the bortezomib arm vs. 66% for the

dexamethasone arm, which represented a 41% decreased relative risk of death in the first year with bortezomib (*P*=.0005). In patients who had received only one prior line of treatment, the probability of survival at one year was 89% for the bortezomib arm vs. 72% for the dexamethasone arm, which represented a 61% decreased relative risk of death in the first year with bortezomib (*P*=.0098). (Richardson et al., 2005)

The optimal schedule for bortezomib is unclear. To further investigate the schedule, a study was recently presented at the American Society of Hematology in 2005.

Patients were indolent lymphoma were randomized to bortezomib 1.3 mg/m^2 on days 1, 4, 8, and 11 of a 21-day cycle (Arm A) or 1.6 mg/m^2 on days 1, 8, 15, and 22 of a 35-day cycle (Arm B) for up to 15 weeks (5 and 3 cycles in arms A and B, respectively). Starting from day 1, rituximab 375mg/m^2 was administered weekly for 4 weeks. In this study, 81 patients were enrolled. At data reporting 46 (23 each arm) had received study drug, and 42 (20 Arm A, 22 Arm B) were evaluable for response. The median bortezomib dose received was 15.9 mg/m^2 (61% of max. expected) in Arm A, and 19.0 mg/m^2 (99% of max. expected) in Arm B. Overall response rates were similar in both arms with 50% Arm A and 45% Arm B. Treatment was tolerated in both arms with grade ≥3 AEs in 10 patients in Arm A and 5 patients in Arm B. The most common grade ≥3 AEs were gastrointestinal toxicities, neutropenia, thrombocytopenia and peripheral neuropathy, but no grade ≥3 thrombocytopenia or neutropenia were observed in Arm B. These results suggest that weekly dosing is less toxic. However, it is unclear if it is as effective because rituximab was administered in both arms and may have obscured differences in outcome. Because the twice weekly schedule has been shown to be effective in mantle cell lymphoma, it will be used in the maintenance arm of this study. Because this is a maintenance and not treatment schedule, patients will receive bortezomib 1.3 mg/m^2 weekly on days 1, 4, 8 and 11 of an 8-week treatment cycle.

1.2.5 DA-EPOCH-bortezomib background

We recently commenced a study, using bortezomib in combination with DA-EPOCH in patients with relapsed or refractory diffuse large B-cell NHL. To reach the optimal dose of Bortezomib used in conjunction with DA-EPOCH, there was an accelerated dose escalation of Bortezomib with doses of 0.5, 1, 1.5 and 1.7 mg/m^2. On the basis of tolerability and feasibility, the 1.5mg/m^2 dose was the dose selected for Phase II evaluation with DA-EPOCH and was administered on days 1 and 4 of a 21-day cycle. Thus far, the toxicities of this combination have included nausea/vomiting, stomatitis, diarrhea, constipation, thrombocytopenia, neutropenia, fever and neutropenia and neuropathy.

Preliminary experience with DA-EPOCH-RB has been obtained in the first 5 patients on the current study. Of these patients, bortezomib doses were reduced or discontinued in all 5 patients due to at least grade 2 neuropathy. These results indicate that the acceptable phase II dose of bortezomib with DA-EPOCH-R should be reduced to 1.3 mg/m^2. The apparent higher toxicity in the current study compared to our previous study may in part be due to the administration of Part A where patients receive bortezomib 1.5 mg/m^2 on days 1, 4, 8, 11 for analysis of translational endpoints and response. Because bortezomib neurotoxicity is cumulative, neurotoxicity during DA-EPOCH-RB will be higher due to bortezomib exposure on Part A. Hence, to reduce neurotoxicity, Part A will administer bortezomib at 1.3 mg/m^2 for days 1 and 4 only (times during which translational endpoints are obtained). As noted above, maintenance bortezomib

will be administered using the standard dose and schedule (bortezomib 1.3 mg/m^2 days 1, 4, 8, 11) but will be repeated at 8-week intervals.

Toxicity of DA-EPOCH-B from our phase I study are shown in the table below.

Toxicity Profiles :DA -EPOCH-Bortezomib*

	Grade			
Adverse Event	2	3	4	**≥ Grade 2 %**
Nausea/vomiting	**2**	**1**	**-**	**8**
Stomatitis	**10**	**-**	**-**	**26**
Constipation	**4**	**-**	**-**	**10**
Diarrhea	**3**	**1**	**-**	**10**
Hematological				
Thrombocytopenia	**7**	**14**	**-**	**54**
Transfusion platelets	**-**	**7**	**2**	**23**
Neutropenia	**3**	**7**	**21**	**79**
Infectious				
Fever and neutropenia	**-**	**6**	**-**	**15**
Neuropathy * *	**1**	**-**	**-**	
Cardiac	**-**	**2**	**-**	**5**

*** * Toxicity is by patient and not cycle** *** Based on 39 cycles**

1.2.6 DA-EPOCH-R background

DA-EPOCH-Rituximab (DA-EPOCH-R) is a novel combination of chemotherapy and the CD-20 monoclonal antibody, rituximab. DA-EPOCH in combination with rituximab has produced a 92% complete remission rate in patients with newly diagnosed mantle-cell lymphoma.[33]

This study sets out to evaluate the combination of EPOCH-Rituximab-Bortezomib induction followed by either early or delayed Bortezomib maintenance in untreated MCL. We plan to accrue 80 patients on this study. This design will allow an assessment of the efficacy of maintenance Bortezomib on time to treatment failure and will provide additional information on the efficacy of Bortezomib at the time of relapse, if this were to occur. In a recent study of EPOCH-R followed by idiotype vaccine in 26 untreated MCL patients, 92% achieved a complete remission following EPOCH-R alone.[33] Additionally, there was 24-month median response duration and 100% survival, indicating that EPOCH-R is a highly effective treatment platform. However, despite the high CR rate, 40% of patients on the study have progressed, suggesting that this approach will not be curative in most patients. Based on the biology of MCL, the addition of Bortezomib to EPOCH-R may further increase the cytotoxicity of the regimen. Additionally, given the over-expression of cyclin D1 in MCL, which is believed crucial in driving cells from the G1 to the S phase, it is worthwhile targeting this pathway through long-term proteasome inhibition. Hence, we propose using maintenance Bortezomib for 18 months following induction with EPOCH-R-Bortezomib. Maintenance therapy has previously been shown to be highly

effective in recurrent Hodgkin's lymphoma and provides different kinetics of cell kill compared to cyclic treatment. We plan to incorporate micro-array analysis of tissue from patients with MCL and correlate gene expression signatures to other molecular and histological markers and to survival and response. This will be achieved by taking advantage of a "window of opportunity" in which all patients will receive a single cycle of Bortezomib before induction with paired tumor samples for microarray and CT scan assessment of tumor response. Recent work by Louis Staudt's group has shown that the proliferation gene expression signature is a quantitative integrator of oncogenic events that predicts survival in MCL and may provide an important prognostic tool in addition to discovering new molecular predictors of outcome using this novel approach.[34] Furthermore, there is little information on the biological effects of Bortezomib in mantle cell lymphoma. Paired samples for analysis by microarray and proteomics will permit further characterization of the potential mechanisms of Bortezomib. These results will be correlated with clinical outcome as a secondary objective.

1.2.7 Microarray Profiling and Proteomics Background

Gene expression profiling on microarrays. We aim to perform gene expression profiling on Affymetrix whole genome arrays in both biopsies from all patients enrolled in the study. We chose gene expression profiling to assess the molecular changes induced by a proteasome inhibitor because gene expression profiling provides a comprehensive and highly quantitative overview of tumor biology and can be carried out even from small amounts of tissue. Gene expression signatures that relate to specific cellular functions or to the activity of distinct signaling pathways can be reproducibly monitored and can identify subsets of patients with distinct tumor biology, different prognosis or differential response to therapy. For example, Rosenwald et al have recently identified a proliferation gene expression signature in a retrospective study in MCL biopsies profiled on the Lymphochip[34]. This proliferation signature integrates different oncogenic events and confers powerful prognostic information. Therefore, we will use the proliferation signature to determine the effect of proteasome inhibition on proliferation rate in vivo and to test whether our proposed combination therapy is able to overcome the adverse prognostic impact of high tumor proliferation. In addition to monitoring changes in gene expression induced by bortezomib, we will use the pre-treatment gene expression profile to develop a molecular predictor of treatment responsiveness. In the second biopsy on bortezomib monotherapy we will use gene expression profiling to characterize sequelae of proteasome inhibition in vivo **to** better define MCL pathobiology and to identify predictors of treatment response. The mechanisms of bortezomib induced cell death and the reason for its apparent tumor selectivity are currently incompletely understood. In vitro data provide a number of hypotheses including inhibition of the NF-kB pathway, activation of caspases, down regulation of cyclin D1 or upregulation of cell cycle inhibitors such as $p21^{cip}$ and $p27^{kip-1}$. The gene expression profiles from the paired biopsies will be helpful in testing whether some of these in vitro observations translate into clinical reality. For instance, we will be able to follow changes in NF-kB regulated genes and thereby estimate the relative importance of this signaling pathway for MCL.

The genetic aberrancies which characterize lymphoma are manifested functionally by changes at the protein level and result in modulation of critical cell signaling pathways such as those involving apoptosis. The technology to profile these protein pathways has evolved through the development of reverse phase protein microarrays (RPPMs)[35-37]. This approach allows not only

quantitation of proteins of interest, but also determination of their activation state, regulated through post-translational modifications such as phosphorylation or cleavage.

The small molecule inhibitor of the proteasome, bortezomib, promotes apoptosis; this effect appears to be due in part to prevention of NF-κB activation, but the precise mechanisms have not been fully established[38]. Furthermore, the proteasome degrades proteins with a wide variety of cellular functions other than regulation of apoptosis (e.g. cell cycle proteins). While previous studies have shown clinical promise of bortezomib in lymphoma, including MCL[39], the key signaling pathways modulated in responders and non-responders have not been characterized. Because bortezomib acts by inhibiting proteasomal degradation, its principal effects are expected to be observed at the protein level.

In this portion of the study we will profile the activation status of cell signaling pathways in MCL before and after bortezomib therapy using RPPMs. Tissue biopsies will be processed by freezing tissue (approximately 0.5 cm^3 for open biopsies or 1 core for needle biopsies) in OCT. Tumor lysates will be immobilized in serial dilutions on a solid phase. Antibodies will be applied in solution phase and binding will be detected by secondary tagging and amplification. Over 400 such antibodies, including those specific for post-translational modifications such as phosphorylation, have been validated for use in RPPMs by the NCI-FDA Clinical Proteomics Program. The precision, sensitivity and linearity of this protocol have been validated using clinical specimens[35]. Bioinformatic analysis of the data can identify significant proteins or combinations, including the hierarchical clustering typical of microarrays[35]. The Center for Cancer Research, National Cancer Institute, currently has open clinical trials for other diseases that incorporate this method of RPPMs to evaluate protein expression in pre- and post-treatment tissue biopsies.

The goal of these analyses will be (1) to identify protein profiles (including activation status of specific signaling pathways) from pre-treatment specimens that correlate with response to bortezomib in MCL; and (2) to determine differences between pre- and post-treatment protein profiles that correlate to response. These data will be helpful in:

(1) Development of future clinical trials of individualized therapy, including:
    a. Selective use of bortezomib in patients with appropriate pre-treatment protein profiles.
    b. Selective continuation of bortezomib (vs. change in therapy) based on proteomic correlates of response.

(2) Identification of activation of undesirable (e.g. anti-apoptotic) pathways as an inadvertent effect of proteasome inhibition, particularly in non-responders. This information could be used to:
    a. Suggest targeted therapies which might be used successfully in combination with bortezomib.
    b. Direct development of novel, more selective, proteasome inhibitors.

1.2.8 Genomic Methylation Analysis Background

Epigenetic changes including aberrant methylation of gene promoters and acetylation of histones have been shown to regulate gene transcription in normal and cancer cells. Gene expression profiling has identified groups of genes associated with resistance to the proteasome inhibitor Bortezomib (BZM), a promising novel therapy for Mantle Cell Lymphoma(MCL). We have

developed in-house, a high resolution, comprehensive global methylation assay called HELP (HPA II Enzyme Ligation mediated PCR amplification). Briefly, genomic DNA is digested using 2 restriction enzymes (HPAII and MSP1) that are iso-schizomers of each other, the resulting products amplified using ligation-mediated PCR and hybridized onto a custom-designed promoter array using the Nimblegen platform. Preliminary data comparing MCL cell line specimens to Naïve B cells using HELP shows abnormal methylation of several key genes like p53 and NPM1. We hypothesize that aberrant epigenetic silencing may regulate genes associated with Bortezomib resistance in MCL. Integrative genomic approaches combining array- based comparative genomic hybridization (CGH) and gene expression have identified novel deletions involving key molecules in MCL biology. We will determine the aberrantly methylated genes involved in MCL pathogenesis by comparing these results with normal mantle zone B cells from routine tonsillectomy specimens. Moreover, by correlating methylation profiles to clinical response in this study we will develop a prediction model for BZM resistance, which could be used in future large scale clinical trials to individualize therapy in MCL. Leftover genetic material (gDNA) will be used to confirm the HELP results at individual genomic loci using MassArray, which is a high throughput Robotic Mass-spectrometric analysis system specifically designed for measuring small amounts of DNA and RNA.

2 ELIGIBILITY ASSESSMENT AND ENROLLMENT

2.1 ELIGIBILITY CRITERIA

2.1.1 Diagnosis of mantle cell lymphoma (confirmed at NCI). All variants are eligible.

2.1.2 Age ≥ 18 years.

2.1.3 No prior treatment except for local radiation or a short course of steroids for control of symptoms. 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.

2.1.4 All stages of disease.

2.1.5 ECOG performance status ≤ 3.

2.1.6 Adequate major organ function (serum creatinine ≤1.5 mg/dl or creatinine clearance > 50 ml/min; bilirubin ≤ 1.5 mg/dl (total) except < 5 mg/dl in patients with Gilbert's syndrome as defined by > 80% unconjugated; ANC > 1000 and platelets > 75,000) unless impairment due to organ involvement by lymphoma.

2.1.7 No myocardial infarction within 6 months prior to enrollment or New York Hospital Association (NYHA) Class III or IV heart failure, 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.

2.1.8 No grade 2 ≥ peripheral neuropathy within 14 days before enrollment.

2.1.9 Ability to give 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.

2.1.10 HIV antibody negative.

2.1.11 Female subject is either post-menopausal at least 1 year before the Screening visit or surgically sterilized or if they are of childbearing potential, agree to practice 2 effective methods of contraception (i.e., a hormonal contraceptive, intra-uterine device, diaphragm with spermicide, condom with spermicide, or abstinence) at the same time, from the time of signing the informed consent through 30 days after the last dose of study treatment, or agree to completely abstain from heterosexual intercourse. Female subject is not 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

2.1.12 Male subject even if surgically sterilized (i.e., status post vasectomy) must agree to practice effective barrier contraception during the entire study treatment period and through 30 days after the last dose of study treatment, or agree to completely abstain from heterosexual intercourse.

2.1.13 No invasive tumors within the last 5 years unless confined to an organ (e.g. prostate or thyroid cancer) and treated with curative therapy (e.g. surgery and/or radiation). Please note, there must be no evidence of the prior malignancy using standard criteria to evaluate the specific prior malignancy.

2.1.14 No known involvement of central nervous system by lymphoma

2.1.15 No history of hypersensitivity to bortezomib, boron or mannitol.

2.1.16 Patient has not received other investigational drugs with 14 days before enrollment.

2.1.17 No serious medical or psychiatric illness likely to interfere with participation in this clinical study.

2.1.18 Exclusion for FDG scan is anyone exceeding the weight limit of the scanner (350 lb).

2.2 EVALUATION PRE-TREATMENT

To be done within 4 weeks of commencing treatment; 2.2.5 must be done within one week of commencing treatment:

2.2.1 Complete History and Physical examination

2.2.2 CBC, differential, PT, PTT, AST, ALT, LDH, alkaline phosphatase, bilirubin, albumin, calcium, phosphate, uric acid, creatinine (24-hour creatinine clearance if serum creatinine

> 1.5 mg/dl), electrolytes, urinalysis, immunoglobulin free light chains and serum protein electrophoresis. Type & screen, and isohemagglutinin titer.

2.2.3 HIV antibody, Anti-HCV antibody, & hepatitis B surface antigen.

2.2.4 HLA typing (A,B, Cw) and Anti-varicella zoster virus IgG ELISA

2.2.5 HCG (serum) in women of childbearing potential.

2.2.6 Electrocardiogram

2.2.7 MUGA or echocardiogram in patients with history of MI or CHF.

2.2.8 Staging: CT scan of chest, abdomen and pelvis; PET (fluorine 18-FDG) scan, bone marrow biopsy. Imaging of head and CSF analysis if clinical suspicion of CNS involvement. Colonoscopy recommended if not performed in previous 2 months and/or known to be positive.

2.2.9 Peripheral blood flow cytometry

3 PARTICIPANT REGISTRATION AND STATUS UPDATE PROCEDURES

Registration and status updates (e.g., when a participant is taken off protocol therapy and when a participant is taken off-study) will take place per CCR SOP ADCR-2, CCR Participant Registration & Status Updates found here.

Randomization takes place after Cycle 6 restaging is completed (weeks 0-12 after completion of cycle 6 of DA-EPOCH-RB). Only patients who have achieved a PR or CR will be eligible for randomization.

4 STUDY IMPLEMENTATION

4.1 STUDY DESIGN

Part A: Bortezomib on days 1, 4, 8 & 11 with sequential tumor sampling. Part A is completed on day 21-28. In patients with leukemic MCL, timed blood samples may be obtained throughout the first course, typically pre and at 6 hours and 24 hours after the first dose and before and 24 hours after the second dose. Select patients without leukemic disease may have serial blood draws during Part A to serve as a comparison group. In patients with nodal disease two lymph node biopsies will be obtained if nodal disease is safely accessible: one pre-treatment and the second 12-24 hours after the 2nd bortezomib dose. On treatment bone marrow aspirate or lymphapheresis to obtain lymphoma cells may be substituted for the lymph node biopsy in select patients.

If Platelet Count <25 K/mcL and impairment is due to organ involvement by lymphoma, proceed to Part B. If a patient has advanced disease that requires urgent treatment with chemotherapy, proceed to Part B.

Part B: Induction: DA-EPOCH-RB x 6 cycles

Part C: Randomization to bortezomib maintenance versus observation for patients who have achieved PR or CR. Randomization will occur within 12 weeks after completion of Part B (Post-cycle 6 of DA-EPOCH-RB). Patients with disease progression in the observation arm will be offered bortezomib treatment. Patients cannot be randomized to maintenance if they

have any grade of toxicity which forbids bortezomib administration. Toxicity must resolve to a lower grade (that allows administration of bortezomib) within 12 weeks of completing DA-EPOCH-RB to be eligible for randomization. If toxicity does not resolve to this level within 12 weeks, the patient is not eligible for randomization.

4.2 DRUG ADMINISTRATION

- **Part A: Bortezomib x 1 cycle (21 days)**

Agent	Dose	Route	Duration	Schedule
Bortezomib	1.3 mg/m^2	IV injection	Bolus (3-5 sec)	Days 1, 4, 8, 11

- **Part B: DA-EPOCH-Rituximab-Bortezomib. (Begin after part A day 21 to 28).**

  All patients initiate therapy at Dose Level 1 of DA-EPOCH-RB shown below. Doses are based on actual body weight for all patients. Future dose adjustments based on hematological toxicity as shown below.

Drugs	**Dosages & Administration/Schedule**
Bortezomib	1.3 mg/m^2 per dose for 2 doses on day 1 (before rituximab) and day 4 (between etoposide+doxorubicin+vincristine bag exchanges)
Prednisone	60 mg/m^2 PO BID days 1-5 (first dose at least 60 min before starting rituximab)
Rituximab	375 mg/m^2 day 1 (before etoposide+doxorubicin+vincristine infusion begins; see Section **13.2** for administration instructions)
Etoposide	50 mg/m^2/day CIV days 1- 4 (96 hour infusion)
Doxorubicin	10 mg/m^2/day CIV days 1-4 (96 hour infusion)
Vincristine	0.4 mg/m^2/day CIV days 1-4 (96 hour infusion)
Cyclophosphamide	750 mg/m^2 IV day 5 over 30-60 mins
Filgrastim	Body weight <75 kg: 300 mcg/dose Body weight ≥75 kg: 480 mcg/dose Doses are given once daily by subcutaneous injection on days 6 to 15 or ANC > 5000/mcL after the leukocyte nadir.
Cycle Length	Repeat cycle every 21 days

- Repeat cycles every 21 days. Delay cycle until ANC > 1000/mcL or platelets >75,000/mcL. Use filgrastim to increase ANC and begin next cycle as soon as ANC recovers. If no recovery after 2 weeks, contact study Chair for guidance.

4.2.1 Dose Adjustments Based on Hematological Toxicity for DA-EPOCH-RB

Doses for doxorubicin, etoposide and cyclophosphamide will be based on measurements of the previous cycle ANC or platelet nadir whichever is lower. **Dose adjustment is based on measurements of <u>twice weekly CBC only</u>, even if additional CBCs are obtained. Twice weekly CBCs must be at least 3 days apart.**

- If Nadir ANC ≥ 500/mcL on all measurements: ↑ One level above last cycle
- If Nadir ANC < 500/mcL on 1 or 2 measurements: Same level as last cycle
- If Nadir ANC < 500/mcL ≥ 3 measurements: ↓ One level below last cycle

Or

- If nadir platelet < 25,000/mcL on ≥ 1 measurement: ↓ One level below last cycle

4.2.2 Dose Levels

Adjustments apply only to etoposide, doxorubicin and cyclophosphamide. Levels below 1 only involve 20% reductions in cyclophosphamide.

Drugs	Drug Doses per Dose Levels							
	-2	-1	**1**	2	3	4	5	6
Doxorubicin (mg/m^2/day)	10	10	**10**	12	14.4	17.3	20.7	24.8
Etoposide (mg/m^2/day)	50	50	**50**	60	72	86.4	103.7	124.4
Cyclophosphamide (mg/m^2)	480	600	**750**	900	1080	1296	1555	1866

4.2.3 Part C: Bortezomib maintenance versus observation (followed by bortezomib if progression occurs).

Patients must have achieved at least a PR after DA-EPOCH-RB to be randomized on Part C. Randomization takes place when patient is eligible after Cycle 6 restaging is completed. Patients cannot be randomized to maintenance if they have any grade of toxicity which forbids bortezomib administration. Toxicity must resolve to a lower grade (that allows administration of bortezomib) within 12 weeks of completing DA-EPOCH-RB to be eligible for randomization. If toxicity does not resolve to this level within 12 weeks, the patient is not eligible for randomization.

4.2.4 Maintenance bortezomib arm

Begin maintenance 8-12 weeks after completion of DA-EPOCH-RB (e.g. Day 77 of last cycle). Bortezomib 1.3 mg/m^2 days 1, 4, 8 and 11 every 56 days (i.e. q8 weeks) for 18 months (10 cycles) or until disease progression, whichever comes first. After maintenance, begin follow-up as described in Section **4.5.2**.

Agent	Dose	Route	Duration	Schedule
Bortezomib	1.3 mg/m^2	IV injection	Bolus (3-5 sec)	Days 1, 4, 8, 11 Repeat q 56 days

4.2.5 Observation (followed by bortezomib if progression occurs) arm

Observation continues for 18 months or until disease progression, whichever comes first. If disease progression occurs on the observation arm, begin bortezomib. Bortezomib 1.3 mg/m^2 days 1, 4, 8, and 11 every 28 days (i.e. q4 weeks). Continue treatment for 18 months or until disease progression, whichever comes first. After completion of observation or treatment begin follow-up as described in Section **4.5.2**.

Agent	Dose	Route	Duration	Schedule

Bortezomib	1.3 mg/m^2	IV injection	Bolus (3-5 sec)	Days 1, 4, 8, 11 Repeat q 28 days

4.3 TREATMENT MODIFICATIONS

4.3.1 Part A: Bortezomib toxicity reasonably ascribed to treatment

4.3.1.1 Hematological Toxicity

Day 1 platelets < 25,000/mcL	Proceed to Part B with DA-EPOCH-RB.
Day 4 platelets < 25,000/mcL	Stop bortezomib and proceed to Part B with DA-EPOCH-RB 10-14 days after last bortezomib dose

4.3.1.2 Diarrhea Toxicity

At first loose stool, start loperamide 2 mg po q 2 h while awake and q 4 h while sleeping. Continue around the clock until 12 h diarrhea free. If diarrhea free > 12 h, stop loperamide. If new episode, retreat as outlined. Please note the manufacturer's recommended maximum daily dose of loperamide is 16 mg. If grade 3 diarrhea accompanied by mucus or dehydration, stop bortezomib and proceed to Part B with DA-EPOCH-RB 10-17 days after last bortezomib dose

4.3.2 Part B: DA-EPOCH-RB toxicity reasonably ascribed to treatment

4.3.2.1 Hematologic Toxicities

See Section **4.2.1** Part B Dose Adjustments for EPOCH-RB

4.3.2.2 Ileus and Constipation

Symptomatic ileus/constipation may occur. Because the severity of constipation is dose related, it is usually unnecessary to stop the vincristine altogether. Every effort should be made to not unnecessarily reduce vincristine doses. Ileus/constipation is usually worse on the first cycle, so prophylactic bowel care is essential. If vincristine dose is reduced for this toxicity, it can often be increased to full dose on subsequent cycles without recurrence of severe ileus/constipation. If ileus or constipation requires hospitalization, reduce vincristine 25%. If symptoms resolve after vincristine reduction, increase dose to previous level on subsequent cycles.

4.3.2.3 Neurological Toxicity

Sensory or Motor Neuropathy

Neurotoxicity Grade	**Vincristine mg/m^2/day**	**Bortezomib mg/m^2/day**
1 without neuropathic pain	0.4 mg/m^2/day	1.3 mg/m^2/day
1 with neuropathic pain	0.2 mg/m^2/day	1.3 mg/m^2/day
2 without neuropathic pain	0	1.3 mg/m^2/day
≥ 2 with neuropathic pain	0	0

If neuropathy resolves to a lower grade, doses for that lower grade may be reinstituted at investigator discretion. If the grade of neuropathy increases after being re-escalated, doses must be reduced for the appropriate toxicity grade and may not be re-escalated, even if neuropathy resolves again to a lower grade.

4.3.2.4 Hepatic Toxicity

Patients with mild hepatic impairment (bilirubin ≤ 1.5 x 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 ≥ Grade 2 (> 1.5 -3.0 X ULN) while on study, the investigator should hold bortezomib until the toxicity returns to < Grade 2. Restarting bortezomib at the next lower dosed level could be considered at the Investigator's discretion and following exclusion of bortezomib-induced liver impairment and careful consideration of liver disease due to other causes, such as, but not limited to, active infection and mantle cell-related liver disease.

No doxorubicin dose modifications for increased bilirubin. Pharmacokinetic data from our group has shown no significant effect of bilirubin on doxorubicin clearance. The following are the dose reductions that apply to vincristine for hyperbilirubinemia due to disease. NOTE: INCREASE VINCRISTINE TO FULL DOSE AFTER BILIRUBIN NORMALIZES

Total bilirubin on day 1	**Vincristine mg/m^2/day**
1.5-3 mg/dL	0.3 mg/m^2/day
> 3.0 mg/dL	0.2 mg/m^2/day

4.3.2.5 Diarrhea Toxicity

Diarrhea treatment ascribed to Bortezomib: At first loose stool: Start loperamide 2 mg p.o. q 2 h while awake and q 4 h while sleeping. Continue around the clock until 12 h diarrhea free. If diarrhea free > 12 h, stop loperamide. If new episode, retreat as outlined. If grade 3 diarrhea accompanied by mucus or dehydration, hold doses of bortezomib (if applicable) and hydrate.

Diarrhea management for next cycle dosing:

Diarrhea Grade	**Bortezomib mg/m^2/day**
≥ grade 3 or associated with mucus or dehydration	1 mg/m^2/day

4.3.2.6 Other Non-Hematological Toxicity

Toxicity Grade	**Management**
≥ grade 3	Hold bortezomib until ≤ grade 1. Restart bortezomib at: 1 mg/m^2/day. If recurrence of ≥ grade 3 toxicity, hold bortezomib until ≤ grade 1. Restart bortezomib at 0.75 mg/m^2/day. If recurrence of ≥ grade 3 toxicity, discontinue bortezomib during DA-EPOCH-RB.

4.3.2.7 Infusion Related Toxicity

Side effects of rituximab may be infusion rate related and may be reduced by slower administration or premedication. Thus, dose reductions of rituximab will not be made. Rituximab will be discontinued for the duration of the cycles in patients with grade 4 allergic reactions. At

the discretion of the PI, rituximab may be administered on the following cycles using slower infusion rates and pretreatment with diphenhydramine using standard medical practice.

4.3.3 Part C. Maintenance Bortezomib or Treatment after Observation toxicity reasonably ascribed to treatment

Patients cannot be randomized to maintenance if they have any grade of toxicity which forbids bortezomib administration. Toxicity must resolve to a lower grade (that allows administration of bortezomib) within 12 weeks of completing DA-EPOCH-RB to be eligible for randomization. If toxicity does not resolve to this level within 12 weeks, the patient is not eligible for randomization. The dose modifications apply to the first and all subsequent maintenance cycles.

4.3.3.1 Hematological Toxicity

Day 1 platelets < 25,000/mcL	Delay until recovery above this level
*Days 4, 8 or 11 platelets < 25,000/mcL	Hold dose for cycle.

*Draw CBC on these days only if day 1 platelets <50, 000/mcL or as clinically indicated.

4.3.3.2 Diarrhea Toxicity

Diarrhea treatment during cycle: At first loose stool: Start loperamide 2 mg po q 2 h while awake and q 4 h while sleeping. Continue around the clock until 12 h diarrhea free. If diarrhea free > 12 h, stop loperamide. If new episode, retreat as outlined. Please note the manufacturer's recommended maximum daily dose of loperamide is 16 mg. If grade 3 diarrhea accompanied by mucus or dehydration, hold doses of Bortezomib and hydrate.

Diarrhea management for next cycle dosing:

Diarrhea Grade	**Bortezomib mg/m^2/day**
≥ grade 3 or associated with mucus or dehydration	1 mg/m^2/day

4.3.3.3 Neurotoxicity

Sensory or Motor Neuropathy

Neurotoxicity Grade	**Bortezomib Management**
Grade 1	Bortezomib 1.3 mg/m^2
Grade 2 without pain (interfering with function but not with activities of daily living).	Bortezomib 1 mg/m^2
Grade 2 with pain or grade 3 (interfering with activities of daily living)	Hold bortezomib until ≤ grade 2 without pain. Reduce bortezomib to 1 mg/m^2 and administer cycles every 12 weeks. If toxicity ≥ grade 2 with pain after 12 weeks, discontinue bortezomib.
Grade 4 (disabling)	Discontinue bortezomib

If neuropathy resolves to lower grade, doses for that lower grade may be reinstituted at investigator discretion. If the grade of neuropathy increases after being re-escalated, doses must be reduced for the appropriate toxicity grade and may not be re-escalated, even if neuropathy resolves again to a lower grade.

4.3.3.4 Other Non-Hematological Toxicity

Toxicity Grade	**Management**
≥ grade 3	Hold bortezomib until ≤ grade 1. Restart bortezomib at 1 mg/m^2/day. If recurrence of ≥ grade 3 toxicity, hold bortezomib until ≤ grade 1. Restart bortezomib at 0.75 mg/m^2/day. If recurrence of ≥ grade 3 toxicity, discontinue bortezomib.

4.4 ON STUDY EVALUATION

4.4.1 Part A: Bortezomib alone

Studies	Pre-therapy[A]	Day -1 or day 1 of cycle	Day 5 (12-24 hours after second bortezomib dose	Day 4 of cycle
Hx; PE; VS; PS	x			
Tumor Measurement	x			
CBC/diff	x	x	x	x
Electrolytes, Creatinine, BUN, ALT, AST, Bilirubin, LDH, Ca^{++}, Phos, Mg	x	x	x	x
EBV viral load	x			
Immunoglobulin Free Light Chains, Serum Protein Electrophoresis, Isohemagglutinin titer, Type & Screen	x			
TBNK, 24 cc red & green CPT tubes	x			
HLA typing, Anti-varicella zoster virus	x			
CT chest/abd/pelvis, PET scan[D]	x			
Research PET scan			x[E]	
Bone marrow biopsy & aspirate, peripheral blood flow cytometry	x			
Colonoscopy[B]	x			
Optional Tumor biopsy, timed blood collections and/or apheresis[C]	x		x[C]	
10 cc red top for serum storage	x			

A- Initial assessment is to be performed within 4 weeks prior to starting treatment.

B- Colonoscopy is recommended pre-treatment unless prior colonoscopy within 2 months or known to be positive or medically contra-indicated.

C- Biopsies will be obtained in all patients (who agree to the optional biopsy) with safely accessible lymph nodes. Snap freeze and store tissue biopsies for translational studies. In patients with leukemic MCL treated at the NCI, up to 8 additional blood samples (typically 6 and 24 hrs post dose 1, and pre and 24 hours post dose 2 of bortezomib) of 20-40cc each may be obtained to collect lymphocytes for analysis of gene expression on microarray and proteomic analysis. In these patients a cbc/diff will be obtained at the time of research blood collection. The amount of blood that may be drawn from adult patients for research purposes shall not exceed 10.5 mL/kg or 550 mL, whichever is smaller, over any eight week period. Lymphapheresis may be performed pre-treatment in all patients with leukemic MCL at the NCI and may be repeated 12-24 hours after the first or second bortezomib dose instead of the timed blood draws. Tissue samples are to be stored at -80º C and shipped on dry ice.

D- Clinical PET scans may be performed at other time points throughout this protocol if the investigator deems it medically necessary.

E- If research biopsies are obtained, research PET scan will be within 24 hours of the second biopsy. If no research biopsies are obtained, the PET scan may be omitted. If a patient has evidence of tumor reduction between day 8 and 21, a repeat PET scan may be done at the end of Part A cycle.

4.4.1.1 Microarray and Proteomic analysis

The pretreatment lymph node biopsy (optional) will be used to perform all standard diagnostic tests. In addition we will extract RNA and protein for research studies. The second biopsy (optional) obtained during Bortezomib monotherapy will be used for research studies only. In cases where there is peripheral blood involvement by mantle cells, timed blood collections or apheresis may be carried out in addition to a lymph node biopsy. Standard diagnostic tests will be sent and samples will be taken for microarray analysis as above. Microarray and proteomic analysis may be performed on lymph node biopsies, serum, whole blood samples and apheresis collected for research. In protein extracts of tumor cells pre and during therapy we aim to determine activity of key regulatory proteins such as components of the NF-kappa B signal transduction pathway, the levels of cell cycle inhibitors such as $p21^{cip}$ and $p27^{kip-1}$ and the p53 stress response. In addition, we may determine serum levels of proteins that play a role in lymphocyte function including chemokines and cytokines. Where possible we may initiate studies that aim at characterizing changes in protein levels on a global scale

4.4.1.2 Timing of the second biopsy

The optimal timing for the on-treatment biopsy can only be estimated at this time. Experiments in cell lines indicate that after 6 hours of proteasome inhibition there are many highly significant changes in gene expression. On the other hand, in a recent study on the effect of Fludarabine on chronic lymphocytic leukemia cells maximal effects required at least 24 hours to develop and the most robust response was observed between 48 and 96hours from start of therapy (Rosenwald, 2004). Thus, the optimal time point to detect changes in response to bortezomib could range from 6 hours after the initial infusion to days after the second infusion. We will perform the second biopsy 12-24 hours after the first or second infusion for several reasons: first, the relatively long time interval will make it likely that we will be able to detect effects of proteasome inhibition on signaling pathways such as the NF-kB pathway that may not be immediately interrupted by bortezomib; second, we will be better able to detect cumulative changes in protein levels and third, scheduling the second biopsy at these time points minimizes interference with an orderly treatment cycle. We plan to analyze a first set of biopsies to

determine whether there are any changes in gene expression detectable in these samples. If there are no or only minimal changes in gene expression we will move the biopsy time point. From the timed blood samples in patients with leukemic MCL we will derive a better understanding of the kinetics of molecular changes in the tumor cells on bortezomib therapy. This data will help determine the optimal time point for lymph node biopsies.

4.4.1.3 Tissue immunohistochemistry

- Tumor tissue will be analyzed by immunohistochemistry. RT-PCR may be performed on tumor samples to correlate changes in gene expression.
- Tumor tissue may be stored for future research assays which are related to this study and do not pose an increase in patient risk.

4.4.1.4 Genome–wide Methylation Analysis

Genomic DNA (gDNA) will be prepared and digested using HPAII and MSPI enzymes from coded tumor samples from enrolled MCL patients. Digested DNA products are then amplified using ligation mediated PCR and hybridized to a custom high density oligonucleotide Microarray. Methylation array testing will be done in duplicate and analyzed using an extensive quality control, primary analysis and normalization pipeline based on the R statistical package and developed by statisticians and bio-informaticians of the Einstein Epigenomics program. Data from all patients will be analyzed by supervised clustering to determine differentially methylated genes in MCL and any correlation for promoter methylation to response to bortezomib. We will confirm results at individual loci by mass spectrometric analysis using the MassArray machine.

4.4.1.5 Biopsy Procedure

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

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

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

biopsies are taken from precisely the same location of tumors) could minimize the added noise from the interpretation of already voluminous and noisy data (as in the case of the gene microarrays).

The correlation and anatomic mapping of tumor biology to multiparametric imaging may improve the search for reliable imaging surrogates for tumor biology or behavior. Image fusion with CT/PET or image registration with electromagnetic tracking allows for the biopsies to be performed with multimodality guidance. This allows PET scans or functional dynamic MRI to provide the guidance for biopsy needle placement, such that biopsies may be taken from specific areas of tumor that have higher metabolic activity on FDG PET for example.

We will use precision techniques like automated laser pointers, to facilitate being able to reliably sample a tumor from precisely the same region on repeated biopsies. This should maximize our chances of detecting significant alterations in tumor biology and protein or gene expression signatures (such as the proliferation signature or upregulation of cell cycle inhibitors).

4.4.2 Part B: DA-EPOCH-RB

Studies	Pre-therapy [A]	Day -1 or Day 1 of cycles 2-6	Twice weekly each cycle [F]	End of cycle 4 and 6 (+/- 1 day)	End of cycle 6 (+/- 2 days)
Hx; PE; VS; PS	X	x			
Tumor Measurement	X			x	
CBC/diff	X	x	x		
Electrolytes, Creatinine, BUN, ALT, AST, Bilirubin, LDH, Ca^{++}, Phos, Mg	X	x			
Isohemagglutinin titer, Immunoglobulin Free Light Chains, Serum Protein Electrophoresis,	X				X
EBV viral load					x
TBNK, 24 cc red & green CPT tubes	X				x
Anti-varicella zoster virus					x
CT chest/abd/pelvis	X			x	
Clinical PET scan					x
Peripheral blood flow cytometry, Bone marrow biopsy & aspirate[B]	X				X[B]
Colonoscopy[C]					x
10 cc whole blood for storage[D]	X[E]				x
10 cc red top for serum storage	X	X			X

A. Pre-DA- EPOCH-R-B evaluation.

B. If patient proceeds directly to Part B, without Part A, then peripheral blood flow cytometry and bone marrow biopsy are to be performed pre-treatment. Repeat both tests if positive at diagnosis.

C. Repeat colonoscopy after cycle 6 if positive at initial diagnosis (or not performed) and not medically contraindicated.

D. 10 cc whole blood for DNA extraction. Draw blood in 10 cc tube with EDTA as preservative. Call Dr. Adrian Wiestner's lab for pick-up: 301-451-7135.

E. May be requested in patients who presented with leukemic MCL.

F. Twice weekly (e.g., on Monday & Thursday or Tuesday & Friday to assure that counts are checked every three to four days).

4.4.3 Part C: Bortezomib maintenance versus observation with treatment at progression.

Studies	Pre-Cycle 1	Day 1(+/- 1 day) each cycle	Day 4, 8 and 11 of each cycle[A]	Every 4 months in both arms[B]	Follow up[E, F]
Hx; PE; VS; PS		x		x	x
Tumor Measurement				x	
CBC/diff		x	x	x	x
Electrolytes, Creatinine, BUN, ALT, AST, Bilirubin, LDH, Ca^{++}, Phos, Mg		x		x	x
Immunoglobulin Free Light Chains, Serum Protein Electrophoresis,					x
TBNK, 24 cc red & green CPT tubes[G]				x	
CT chest/abd/pelvis	x[H]			x	x
Colonoscopy[C]					
Bone marrow biopsy & aspirate[C]					
Tumor biopsy and/or apheresis[D]					
10 cc red top for serum storage		x		x	x

A- For patients undergoing treatment on the maintenance arm or in patients with progression on the observation arm. Draw CBC on these days only if Day 1 platelets <50, 000/mcL or as clinically indicated.

B- Restage every 4 months (+/- 1 week) in patients undergoing treatment and in patients on observation during treatment or observation period.

C- A repeat bone marrow biopsy and colonoscopy may be done in patients who progress. Repeat if patients receive bortezomib after progression on the observation arm and only if they achieved a second CR by CT scan or at end of treatment.

D- When possible, a repeat lymph node biopsy and/or lymphapheresis will be obtained in patients who progress after DA-EPOCH-RB.

E- Patients who are not randomized to treatment or observation, follow-up to occur every 4 months (+/- 2 weeks) for 2 years, then every 6 months (+/- 2 weeks) for 2 years, and then yearly (+/- 4 weeks) thereafter. Patients who are randomized to treatment or observation on Part C will proceed to follow-up after completion of treatment or observation. Follow-up to occur every 6 months (+/- 2 weeks) for 2 years, and then yearly (+/- 4 weeks) thereafter.

F- After the sixth year, patients may be followed by local oncologist who agrees to send office visit notes and lab results to Research Team. Records should be sent via fax to Research Nurse Office 301.480.1105, attention Dr. Wyndham Wilson. Patients who are not randomized to treatment or observation on part C will proceed directly to follow up. Patients who are taken off-study will continue to be followed for survival using publicly available information, such as the Social Security Death Index.

G- This test to be done only up to 2 years s/p the end of Part B.

H- Pre-Cycle 1 CT scan may be done up to 2 weeks before beginning Part C.

4.5 POST-TREATMENT EVALUATIONS

4.5.1 Patients who are not randomized to treatment or observation on Part C will proceed directly to follow up. Follow-up to occur every 4 months (+/- 2 weeks) for 2 years, then every 6 months (+/- 2 weeks) for 2 years, and then yearly (+/- 4 weeks) thereafter.

4.5.2 Patients who are randomized to treatment or observation on Part C will proceed to follow-up after completion of treatment or observation. Follow-up to occur every 6 months (+/- 2 weeks) for 2 years, and then yearly (+/- 4 weeks) thereafter.

4.5.3 For all groups: after the sixth year, patients may be followed by local oncologist who agrees to send office visit notes and lab results to Research Team. Records should be sent via fax to Research Nurse Office 301.480.1105, attention Dr. Wyndham Wilson. Adverse event data that occurs during the follow-up period that is unrelated to study treatment will not be reported. Patients who are taken off-study will continue to be followed for survival using publicly available information, such as the Social Security Death Index.

4.5.4 Patients who are lost to follow-up will continue to be followed for survival using publicly available information, such as the Social Security Death Index.

4.5.5 As part of the clinical evaluation and follow up we will use CT scans and Positron Emission Tomography (PET) scan to determine the extent of disease and response to treatment. In addition, we will do one CT scan and up to two PET scans for research purposes to look for any early effect of the VELCADE you will receive. Some patients may also undergo up to two biopsies under CT guidance for research purposes. This radiation is for research purposes only and is not necessary for medical care.

4.6 COST AND COMPENSATION

4.6.1 Costs

NIH does not bill health insurance companies or participants for any research or related clinical care that participants receive at the NIH Clinical Center. If some tests and procedures performed outside the NIH Clinical Center, participants may have to pay for these costs if they are not covered by insurance company. Medicines that are not part of the study treatment will not be provided or paid for by the NIH Clinical Center.

4.6.2 Compensation

Participants will not be compensated on this study.

4.6.3 Reimbursement

The NCI will cover the costs of some expenses associated with protocol participation. Some of these costs may be paid directly by the NIH and some may be reimbursed to the participant/guardian as appropriate. The amount and form of these payments are determined by the NCI Travel and Lodging Reimbursement Policy.

4.7 OFF TREATMENT CRITERIA

- Completion of protocol therapy
- Progressive disease requiring non-protocol therapy
- Participant requests to be withdrawn from active therapy

- Investigator discretion

4.8 OFF STUDY CRITERIA

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

4.8.1 Lost to Follow-up

A participant will be considered lost to follow-up if he or she fails to return for 3 scheduled visits and is unable to be contacted by the study site staff.

The following actions must be taken if a participant fails to return to the clinic for a required study visit:

- The site will attempt to contact the participant and reschedule the missed visit 5 business days and counsel the participant on the importance of maintaining the assigned visit schedule and ascertain if the participant wishes to and/or should continue in the study.
- Before a participant is deemed lost to follow-up, the investigator or designee will make every effort to regain contact with the participant (where possible, 3 telephone calls and, if necessary, a certified letter to the participant's last known mailing address or local equivalent methods). These contact attempts should be documented in the participant's medical record or study file.
- Should the participant continue to be unreachable, he or she will be considered to have withdrawn from the study with a primary reason of lost to follow-up.

5 SUPPORTIVE CARE

5.1 PROPHYLAXIS OF PNEUMOCYSTIS JIROVECI (FORMERLY, CARINII)

All patients will receive prophylaxis for Pneumocystis during EPOCH chemotherapy. Trimethoprim/sulfamethoxazole 1 DS P.O. QD for three days each week. Monday, Wednesday, Friday is the preferred schedule. Patients allergic to either component may receive other standard treatments.

5.2 PROPHYLAXIS FOR HEPATITIS B REACTIVATION.

Patients who are positive for Hepatitis B core antibody (anti-HBc) and not acutely infected are at varying risk for reactivation of Hepatitis B. These patients will have quantitative PCR testing performed for Hepatitis B virus. Additionally, patients at high and moderate risk will receive appropriate prophylaxis for hepatitis B reactivation, e.g., lamivudine 100mg PO daily to continue until 8 weeks after last chemotherapy with repeat quantitative PCR performed 4-8 weeks after stopping prophylaxis.

anti-HBc	HBsAg	Anti-HBs	Risk	HBV PCR	Prophylaxis

+ or −	+	+ or −	High	pre-tx, post 2, 4, 6 cycles	Yes
+	−	−	Moderate	pre-tx, post 2, 4, 6 cycles	Yes
+	−	+	Low	pre-tx, post 3, 6 cycles	No, if PCR neg

5.3 PROPHYLAXIS FOR HERPES SIMPLEX AND/OR HERPES ZOSTER

All patients will receive prophylaxis for Herpes Simplex and/or Herpes Zoster virus infection during Part B EPOCH chemotherapy, using ValAcyclovir 500 mg twice daily, Acyclovir 400 mg twice daily or Famciclovir 500 mg twice daily.

5.4 RECOMMENDATIONS FOR MANAGEMENT OF GASTROINTESTINAL ISSUES:

5.4.1 Prevention and/or treatment of nausea and vomiting prior to and during chemotherapy:

- Ondansetron 24 mg PO x1 dose 30–60 min prior to Cyclophosphamide, followed by Ondansetron 8 mg every 12 hours for 3 more days (5 or 6 doses)
- Prochlorperazine 10 mg PO every 6 hours PRN for nausea or vomiting

5.4.2 Prevention of symptoms of gastroesophageal reflux disease (GERD) and other conditions caused by excess stomach acid:

- Omeprazole 20 mg PO once daily

5.4.3 Prevention and/or treatment of constipation:

- Docusate Sodium 50 mg + Sennosides 8.6 mg 1 tablet PO twice daily.
- Lactulose 10-20 grams (15–30 mL) PO every 6 hours PRN for constipation

6 CORRELATIVE STUDIES FOR RESEARCH

6.1 PROCEDURES FOR COLLECTING TUMOR BIOPSIES AND/OR PERIPHERAL BLOOD CELLS

1. Orders for tumor biopsies, research blood samples and lymphapheresis collections should be placed in CRIS (Clinical Research Information System, Clinical Research Center, NIH, Bethesda, MD)
2. Tumor biopsies will be submitted in native condition to the Department of Pathology, CCR, NIH and handled according to routine procedures. Material released for research studies will be documented on form NIH 2803-1. Initial processing of samples for research will depend on the size of the tumor biopsy. For core biopsies, the research sample will typically consist of 2 cores in a microcentrifuge vial snap frozen on dry ice. Surgical lymph node biopsies may in addition be processed for single cell suspension, additional vials of snap frozen tissue and OCT embedded tissue.
3. Lymphapheresis is performed in the Department of Transfusion Medicine, and blood will be collected in the phlebotomy suite, on a clinical ward, or in an outpatient clinic of the CRC, NIH. Samples will be transferred to the research laboratory at room temperature. Cells will be separated by Ficoll density gradient centrifugation and only mononuclear cells will be harvested, processed, analyzed, and stored.

4. Tumor and normal blood cells may be viably frozen, typically at concentrations of 20-100x10^6/mL in FCS with 10% DMSO using a temperature controlled freezing process to optimize sample viability. Samples will be transferred to Nitrogen tanks for long term storage.
5. Tumor and normal blood cells can be further processed. Additional purification may be carried out by selection with magnetic beads binding to appropriate surface molecules, typically CD19. For analysis cells may be lysed to obtain RNA (using Qiagen manufactured kits are similar) or proteins (salt and/or triton containing buffers with addition of protease and phosphatase inhibitors). Integrity of RNA is monitored by gel electrophoresis and concentration of RNA or protein is measured spectrophotometrically.
6. Research sample inventory and storage. All research samples are assigned a unique number and cataloged. Viably frozen cells are stored in a temperature controlled, alarm secured Nitrogen tank. Tumor biopsies and processed biologic material (RNA, protein) is stored at -80ºC in a temperature controlled, alarm secured -80ºC freezer.

6.2 BIOSPECIMEN COLLECTION

6.2.1 Molecular and Genomic Studies

Samples will be analyzed to assess the effects of treatment on molecular and genetic changes within the cancer genome, and to correlate these with clinical outcomes (e.g., microarray, proteomics, and other genetic and/or mutational analyses). Although direct analysis of germline DNA is not planned, normal genome could be analyzed for comparison with other testing or inadvertently analyzed within other samples, or as part of future analyses.

NOTE: Effective with Amendment O, the option for exploratory genetic testing was added and this protocol amended accordingly, including revised informed consent/procedures.

6.2.2 Methylation/Epigenetic Testing

Coded samples consisting of extracted DNA or cellular material from either frozen blood cells or lymph node biopsies will be sent to Samir Parekh, MD for genome-wide methylation analysis and epigenetic testing. The address to send the samples to is:

Samir Parekh, M.D.
Department of Oncology Hoffheimer 1
Montefiore Medical Center
111 East 210th Street, Bronx, NY 10467
Phone: 718.920.4826

6.2.3 Circulating Tumor DNA

Coded, de-identified frozen or formalin fixed and paraffin embedded (FFPE) human tissue and serum and/or plasma samples will be sent to Adaptive Biotechnologies Corp. It is of research interest to determine if circulating tumor DNA before, during or after therapy is predictive of long-term disease survival. Adaptive Biotechnologies Corp will assess whether immune repertoire data (B-cell immunoglobulin receptor sequences) from the Human Material can be used as biomarkers that correlate with disease-free survival. Adaptive Biotechnologies Corp will use a proprietary method, Immune Cell Receptor Sequencing (ICRS) platform, for amplifying and analyzing immune cell receptor sequences, allowing unprecedented sensitivity and

specificity. Data from experiments conducted by Adaptive Biotechnologies Corp using the Human Material will be provided to NCI and such data provided by Adaptive Biotechnologies Corp to NCI may be used by NCI for any purpose. The address to send the samples to is:

Adaptive Biotechnologies Corp
1551 Eastlake Ave E
Suite 200
Seattle, WA 98102

6.3 SAMPLE STORAGE, TRACKING AND DISPOSITION

All specimens obtained in the protocol are used as defined in the protocol. Samples will be ordered in CRIS and tracked through a Clinical Trial Data Management system. Should a CRIS screen not be available, the CRIS downtime procedures will be followed. Samples will not be sent outside NIH appropriate approvals and/or agreements, if required.

6.3.1 Procedures for stored specimens

- The Clinical Support Laboratory, Leidos Biomedical Research, Inc.-Frederick, processes and cryopreserves samples in support of IRB-approved, NCI clinical trials. All laboratory personnel with access to patient information annually complete the NIH online course in Protection of Human Subjects. The laboratory is CLIA certified for anti-IL15 and certain cytokine measurements, and all laboratory areas operate under a Quality Assurance Plan with documented Standard Operating Procedures that are reviewed annually. Laboratory personnel are assessed for competency prior to being permitted to work with patient samples. Efforts to ensure protection of patient information include:
- The laboratory is located in a controlled access building and laboratory doors are kept locked at all times. Visitors to the laboratory are required to be accompanied by laboratory staff at all times.
- Hard copy records or electronic copies of documents containing patient information are kept in the locked laboratory or other controlled access locations.
- An electronic database is used to store information related to patient samples processed by the laboratory.
- The database resides on a dedicated program server that is kept in a central, locked computer facility.
- The facility is supported by two IT specialists who maintain up to date security features including virus and firewall protection.
- Program access is limited to specified computers as designated by the laboratory director. Each of these computers has a password restricted login screen.
- The database sample entry program itself is accessed through a password protected entry screen.
- The database program has different levels of access approval to limit unauthorized changes to specimen records and the program maintains a sample history.
- Upon specimen receipt each sample is assigned a unique, sequential laboratory accession ID number. All products generated by the laboratory that will be stored either in the laboratory freezers or at a central repository facility are identified by this accession ID.
- Inventory information will be stored at the vial level and each vial will be labeled with both a sample ID and a vial sequence number.

- Vial labels do not contain any personal identifier information.
- Samples are stored inventoried in locked laboratory freezers and are routinely transferred to the NCI-Frederick repository facilities for long term storage.
- Access to stored clinical samples is restricted. Investigators establish sample collections under "Source Codes" and the investigator responsible for the collections, typically the protocol Principal Investigator, specifies who has access to the collection. Specific permissions will be required to view, input or withdraw samples from a collection. Sample withdrawal requests submitted to approved laboratory staff by anyone other than the repository source code owner are submitted to the source code owner for approval. The repository facility will also notify the Source Code holder of any submitted requests for sample withdrawal.
- It is the responsibility of the Source Code holder (generally the NCI Principal Investigator) to ensure that samples requested and approved for withdrawal are being used in a manner consistent with IRB approval.
- The Clinical Support Laboratory does perform testing services that may be requested by clinical investigators including, but not limited to, immunophenotyping by flow cytometry and cytokine testing using ELISA or multiplex platforms.
- When requests are submitted by the NCI investigator for shipment of samples outside of the NIH it is the policy of the laboratory to request documentation that a Material Transfer Agreement is in place that covers the specimen transfer. At a minimum, the lab needs confirmation that one has been executed or an exception was granted from an office authorized to make such exceptions, e.g. NCI Technical Transfer Center. The laboratory does not provide patient identifier information as part of the transfer process but may, at the discretion of the NCI investigator, group samples from individual patients when that is critical to the testing process.
- The NCI investigator responsible for the sample collection is responsible for ensuring appropriate IRB approvals are in place and that a Material Transfer Agreement has been executed prior to requesting the laboratory to ship samples outside of the NIH.

6.3.2 Study Completion, Future Use and Sample Destruction

The study will remain open so long as sample or data analysis continues. Following completion of the planned analyses, samples will remain in storage as detailed above.

Tissue specimens and derived tissue lysates, RNA and DNA collected in the course of this research project may be banked and used in the future to investigate new scientific questions related to this study that are not expressly stated in the present protocol. However, this research may only be done if the risks of the new questions and the proposed research have undergone prospective IRB review and approval. If new risks are associated with the research the Principal Investigator must amend the protocol and obtain informed consent from all research subjects.

If, at any time, a patient withdraws from the study and does not wish for their existing samples to be utilized, the individual must provide a written request. Following receipt of this request, data will be excluded from future distributions, but data that have already been distributed for approved research use will not be able to be retrieved; additionally, the samples will be destroyed (or returned to the patient, if so requested) and reported as such to the IRB.

Any samples lost (in transit or by a researcher) or destroyed due to unknown sample integrity (e.g., broken freezer allows for extensive sample thawing, etc.) will be reported as such to the IRB, the NCI Clinical Director, and the office of the CCR, NCI, as appropriate.

6.4 GENETIC/GENOMIC ANALYSIS

6.4.1 Description of the scope of genetic/genomic analysis

At any point in the analyses, normal genome could be analyzed for comparison with other testing (e.g., mutational analyses, cancer genome).

6.4.2 Description of how privacy and confidentiality of medical information/biological specimens will be maximized

Confidentiality for genetic samples will be maintained as described (Sections **6.3.1** and **6.4.2**). In addition, a Certificate of Confidentiality has been obtained for this study.

6.4.3 Management of Results

Subjects will be contacted if a clinically actionable gene variant is discovered. Clinically actionable findings for this study are defined as disorders appearing in the American College of Medical Genetics and Genomics recommendations for the return of incidental findings that is current at the time of primary analysis. (A list of current guidelines is maintained on the CCR intranet: https://ccrod.cancer.gov/confluence/display/CCRCRO/Incidental+Findings+Lists).

6.4.4 Genetic Counseling

Subjects will be contacted with a request to provide a blood sample to be sent to a CLIA certified laboratory. If the research findings are verified in the CLIA certified lab, the subject will be offered the opportunity to come to NIH to have genetic education and counseling to explain this result; at the time of any such event(s), these activities will be funded by the NCI/CCR in consideration of the specific circumstances. If the subject does not want to come to NIH, a referral to a local genetic healthcare provider will be provided (at their expense).

This is the only time during the course of the study that incidental findings will be returned. No interrogations regarding clinically actionable findings will be made after the primary analysis.

7 DATA COLLECTION AND EVALUATION

7.1 DATA COLLECTION AND EVALUATION

7.1.1 Data Collection

The PI will be responsible for overseeing entry of data into an in-house password protected electronic system and ensuring data accuracy, consistency and timeliness.

All AEs, including clinically significant abnormal findings on laboratory evaluations, regardless of severity, will be followed until return to baseline or stabilization of event.

Document AEs from the first study intervention, Study Day 1, through 30 days after the last dose of the study treatment.

Beyond 30 days after the last intervention, only adverse events which are serious and related to the study intervention need to be recorded.

Primary and final analyzed data will have identifiers so that research data can be attributed to an individual human subject participant.

End of study procedures: Data will be stored according to HHS, FDA and NIH Intramural Records Retention Schedule regulations as applicable.

Loss or destruction of data: Should we become aware that a major breach in our plan to protect subject confidentiality and trial data has occurred, this will be reported expeditiously per requirements in section **8.2.1**.

7.1.2 Data Collection Clarifications

7.1.2.1 Abnormal Laboratory Values

An abnormal laboratory value will be considered an AE if the laboratory abnormality is characterized by any of the following:

- Results in discontinuation from the study
- Is associated with clinical signs or symptoms
- Requires treatment or any other therapeutic intervention
- Is associated with death or another serious adverse event, including hospitalization.
- Is judged by the Investigator to be of significant clinical impact
- If any abnormal laboratory result is considered clinically significant, the investigator will provide details about the action taken with respect to the test drug and about the patient's outcome.

7.2 DATA SHARING PLANS

7.2.1 Human Data Sharing Plan

What data will be shared?

I will share human data generated in this research for future research as follows:

_X_ Coded, linked data in an NIH-funded or approved public repository.

_X_ Coded, linked data in BTRIS (automatic for activities in the Clinical Center)

How and where will the data be shared?

Data will be shared through:

_X_ An NIH-funded or approved public repository: ClinicalTrials.gov.

_X_ Another public repository: dbGaP.

_X_ BTRIS (automatic for activities in the Clinical Center)

_X_ Publication and/or public presentations.

When will the data be shared?

_X_ At the time of publication or shortly thereafter.

7.2.2 Genomic Data Sharing Plan

Unlinked genomic data will be deposited in public genomic databases such as dbGaP in compliance with the NIH Genomic Data Sharing Policy.

7.3 EVALUATION OF CT SCAN MEASUREMENTS

CT Scans are used to assess baseline tumor burden and to determine tumor response. The following measurement guidelines are intended to ensure that tumor measurements and assessments of response are conducted consistently throughout the study.

7.3.1 Part A

Baseline (Pre-treatment) tumor measurements will be obtained using the CT Scan performed prior to the initiation of any Protocol Therapy. These pre-treatment baseline measurements will be recorded and used as the cumulative baseline product when restaging post Part A on cycle 1 Day 21 after the administration of single agent Bortezomib

7.3.2 Part B

The Cumulative Product which was calculated after completion of Cycle 1 Part A will become the baseline tumor measurement for Pre-Part B. This product also serves as the Cumulative Baseline Product for subsequent restaging CT Scans for Part B, i.e. post cycle 4 and Cycle 6.

If it is determined that the patient is to bypass Part A and proceed directly to Part B, then pre-treatment baseline measurements will be obtained and recorded as stated in Part A.

7.3.3 Part C

The Cumulative Product which was calculated after completion of Cycle 6 Part B will become the baseline tumor measurement for Pre-Part C. The CT scan for Pre Part C will be performed when the patient is evaluated for Randomization to Bortezomib maintenance versus observation occurs. This occurs approximately 12 weeks after the completion of Part B. The tumor measurements obtained at this time point will serve as the Cumulative Product for Pre-Part C and subsequent Cumulative baseline products for further treatment and restaging CT Scan evaluations.

7.4 RESPONSE CRITERIA

7.4.1 Response criteria for lymphomas

Responses must last for at least 4 weeks off treatment.

7.4.1.1 Complete response (CR)

Complete disappearance of all detectable clinical and radiographic evidence of disease and disappearance of all disease-related symptoms if present before therapy, and normalization of those biochemical abnormalities (e.g. LDH) definitely assignable to the lymphoma. All lymph nodes must have regressed to normal size (≤ 1.5 cm in greatest diameter if > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in greatest diameter must have decreased to ≤ 1 cm or by more than 75% in the sum of the products of the greatest diameters (SPD). Spleen, if considered to be enlarged before therapy, must have regressed in size and not be palpable on physical examination. The bone marrow must show no evidence of disease by histology. Lymphocyte aggregates within the bone marrow must be negative for B-cell markers (e.g. L26).

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

7.4.1.2 Complete response unconfirmed (CRu)

As per CR criteria except that if a residual node is > 1.5 cm, it must have regressed by > 75% in SPD. Lymphocyte aggregates within the bone marrow must be negative for B-cell markers (e.g. L26).

7.4.1.3 Partial response

≥ 50% decreased in SPD of 6 largest dominant nodes or nodal masses. No increase in size of nodes, liver or spleen and no new sites of disease. Splenic and hepatic nodules must regress by ≥ 50% in the SPD. Bone marrow is irrelevant for determination of a PR.

7.4.1.4 Definition of progressive disease (PD):

Defined by at least one of the following: ≥50% increase in the sum of the products of at least two lymph nodes appearance of new lymph nodes, ≥50% increase in the size of the liver and/or spleen as determined by measurement below the respective costal margin, appearance of new palpable hepatomegaly or splenomegaly that was not previously present, ≥50% increase in the absolute number of circulating lymphocytes.

7.4.1.5 Definition of stable disease

(SD) will be characterized by not meeting any of the criteria outlined above.

7.5 TOXICITY CRITERIA

The NCI Common Toxicity Criteria version 3.0 will be used for toxicity and adverse event reporting. A copy of the CTC version 3.0 can be downloaded from http:/ctep.cancer.gov/reporting/ctc.html. Dose limiting toxicity is defined in section **4.3**.

8 NIH REPORTING REQUIREMENTS / DATA AND SAFETY MONITORING PLAN

8.1 DEFINITIONS

Please refer to definitions provided in Policy 801: Reporting Research Events found here.

8.2 OHSRP OFFICE OF COMPLIANCE AND TRAINING / IRB REPORTING

8.2.1 Expedited Reporting

Please refer to the reporting requirements in Policy 801: Reporting Research Events and Policy 802 Non-Compliance Human Subjects Research found here.

8.2.2 IRB Requirements for PI Reporting at Continuing Review

Please refer to the reporting requirements in Policy 801: Reporting Research Events found here.

8.3 NCI CLINICAL DIRECTOR REPORTING

Problems expeditiously reported to the OHSRP in iRIS will also be reported to the NCI Clinical Director. A separate submission is not necessary as reports in iRIS will be available to the Clinical Director.

In addition to those reports, all deaths that occur within 30 days after receiving a research intervention **s**hould be reported via email to the Clinical Director unless they are due to progressive disease.

To report these deaths, please send an email describing the circumstances of the death to Dr. Dahut at NCICCRQA@mail.nih.gov within one business day of learning of the death.

8.4 NIH Required Data and Safety Monitoring Plan

8.4.1 Principal Investigator/Research Team

The clinical research team will meet on a regular basis when patients are being actively treated on the trial to discuss each patient. Decisions about dose level enrollment and dose escalation if applicable will be made based on the toxicity data from prior patients.

All data will be collected in a timely manner and reviewed by the PI, the study chairman or a lead associate investigator for toxicity. Events meeting requirements for expedited reporting as described in section **8.2.1** will be submitted within the appropriate timelines.

The principal investigator will review adverse events and response data on each patient to ensure safety and data accuracy. The principal investigator will personally conduct or supervise the investigation and provide appropriate delegation of responsibilities to other members of the research staff.

8.5 Procedures for AE and SAE Reporting to Manufacturer

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 bortezomib up to and including 30 days after administration of the last dost of bortezomib. Any SAE that occurs at any time after completion of bortezomib 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 Dr. Wyndham Wilson, 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.

As the study sponsor, Dr. Wilson will be the single point of contact with Millennium for safety reporting. Dr. Wilson will require all local physicians operating under the protocol for all patients (i.e., those from the NCI study site) to report SAEs to Dr. Wilson immediately. Dr. Wilson will be responsible for ensuring that all local physicians are aware of SAE reporting requirements and that they send such reports to Dr. Wilson.

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. 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 (North America Reporting)

Millennium Pharmacovigilance
SAE and Pregnancy Reporting Contact Information
FAX Number: 1-800-963-6290
Email: TakedaOncoCases@cognizant.com

8.6 PROCEDURES FOR REPORTING DRUG EXPOSURE DURING PREGNANCY AND BIRTH EVENTS

If a woman becomes pregnant or suspects that she is pregnant while participating in this study, she must inform the investigator immediately and permanently discontinue study drug. The sponsor-investigator must fax a completed Pregnancy Form (Section **0**) to the Millennium Department of Pharmacovigilance or designee (see Section **8.5**). The pregnancy must be followed for the final pregnancy outcome.

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 (Section **0**) to the Millennium Department of Pharmacovigilance or designee (see Section **8.5**). Every effort should be made to follow the pregnancy for the final pregnancy outcome.

All SAEs that are reported to Millennium must also be forwarded to the Regulatory Affairs Branch, CTEP via email to ctepsupportae@tech-res.com.

9 STATISTICAL CONSIDERATIONS

The primary objective of this study is to determine in a small, randomized study whether there could be a statistically significant difference in progression free survival between patients with mantle cell lymphoma who are randomized to receive EPOCH-RB followed by maintenance Bortezomib versus observation alone.

In order to detect a difference in progression free survival with a two-tailed 0.05 alpha level, simulations were performed to determine the sample size. The following assumptions were made:

Accrual will take place over 24 months. It will be assumed that greater than 90% of patients who enter the trial will experience a PR or CR and hence be eligible for randomization. The observation arm will be assumed to have a median progression free survival of approximately 24 months, and will follow an exponential failure distribution with a 0.0289 hazard rate throughout.

This was based on results obtained from the single arm trial of EPOCH-R + vaccine in mantle cell lymphomas in which the median progression free survival of 26 patients was approximately 24 months, and assuming that the observation arm will have similar results. It will be assumed that the maintenance therapy progression free survival curve will decrease less rapidly than the observation curve, and then plateau. For purposes of calculation, the maintenance therapy curve will be assumed to have an exponential failure rate of ½ that of the other curve for the first 24 months (lambda=0.0144), resulting in approximately 71% PFS at 24 months. It will further be assumed that the curve will flatten out such that it attains 51% PFS by 48 months (lambda=0.0137 from 24 to 48 months) and finally levels out to 50% PFS at 72 months (lambda=0.0008 from 48 to 72 months). Under these assumptions, using 10000 simulations with nQuery Advisor, with 36 patients per arm (total 72), there is 81% power to detect the differences as stated above with a two-tailed 0.05 alpha level test. In order to enroll 72 patients who are able to be randomized, the accrual ceiling will be set at 80 patients, and could be expanded by amendment if necessary to enroll 72 randomized subjects.

The primary evaluation will be a Kaplan-Meier analysis with a two-tailed log rank test. Since accrual is expected to take place rapidly for this trial, and progression free survival probabilities well past 2 years will be of interest to examine, there will be no formal provision for early termination of this trial. Also, because the two arms differ mainly in the use of maintenance therapy vs. standard duration of agents and then observation, it is not expected that there will be differences in toxicity between the two arms. Furthermore, the study will not be blinded and is of limited size. Thus, there will not be a need for formal DSMB evaluation of this study. The PI will monitor the trial in conjunction with the study statistician and will report all adverse events in a timely fashion to the IRB.

Overall survival (OS) will also be determined for both randomized arms of the trial and compared beginning at the date of randomization. Because this is a relatively indolent disease, statistically significant differences in OS will be difficult to observe with only 72 randomized subjects. However, the results obtained will be reported, along with appropriate 95% confidence intervals.

Secondary evaluations will include, but not be limited to the following: Clinical response will be evaluated on all 80 patients who receive the initial cycle of Bortezomib alone, and a 95% confidence interval will be formed about the observed response proportion. Biopsies taken pre-treatment and post this single cycle of Bortezomib will yield gene expression data and microarray data that can be evaluated both to determine if pre-treatment expression levels or gene patterns can be found which differ according to degree of clinical response noted (CR vs. <CR, or CR+PR vs. SD+PD), or to see if changes in expression levels or changes in microarray patterns can be used to predict response to DA-EPOCH-RB. With a total of up to 80 subjects having this data available, even with 90% of patients responding and 10% not responding, there would be over 80% power to detect a 1.2 standard deviation difference between response categories in an individual gene with its expression level evaluated, using a 0.05 alpha level two tailed t-test. Since a large number of such tests may be performed, the power to detect individual differences at that level may be reduced, but since these analyses will be done with exploratory intent, the results obtained will be used to guide future research. Analyses will tend to focus on cell cycle regulatory genes to see if changes in their expression levels and patterns are associated with clinical response, PFS, and OS.

In addition, response and clinical toxicity to DA-EPOCH-RB will be determined and reported descriptively, and a 95% confidence interval about the observed fraction of patients who have a CR or a CR+PR will be formed. As well, time to progression after progressing on the observation arm will be determined using the Kaplan-Meier method. This will be reported descriptively, using appropriate 95% confidence intervals. With 24 months to accrue 80 patients, in order to yield 72 randomized subjects, approximately 3 patients per month are expected to enroll in an average month.

10 COLLABORATIVE AGREEMENTS

10.1 COOPERATIVE RESEARCH AND DEVELOPMENT AGREEMENT (CRADA)

The agent(s) (hereinafter referred to as "Agent(s)"), PS-341, used in this protocol is provided to the NCI under a CRADA with Millennium (hereinafter referred to as "Collaborator(s)") and the NCI Division of Cancer Treatment, Diagnosis and Centers. Therefore, the following obligations/guidelines apply to the use of the Agent(s) in this study:

1. Agent(s) may not be used outside the scope of this protocol, nor can Agent(s) be transferred or licensed to any party not participating in the clinical study. Collaborator(s) data for Agent(s) are confidential and proprietary to Collaborator(s) and should be maintained as such by the investigators.
2. For a clinical protocol where there is an investigational Agent used in combination with (an)other investigational Agent(s), each the subject of different CTAs or CRADAs, the access to and use of data by each Collaborator shall be as follows (data pertaining to such combination use shall hereinafter be referred to as "Multi-Party Data."):
    a. NCI must provide all Collaborators with written notice regarding the existence and nature of any agreements governing their collaboration with NIH, the design of the proposed combination protocol, and the existence of any obligations which would tend to restrict NCI's participation in the proposed combination protocol.
    b. Each Collaborator shall agree to permit use of the Multi-Party Data from the clinical trial by any other Collaborator to the extent necessary to allow said other Collaborator to develop, obtain regulatory approval or commercialize its own investigational Agent.
    c. Any Collaborator having the right to use the Multi-Party Data from these trials must agree in writing prior to the commencement of the trials that it will use the Multi-Party Data solely for development, regulatory approval, and commercialization of its own investigational agent.
3. The NCI encourages investigators to make data from clinical trials fully available to Collaborator(s) for review at the appropriate time (see #5). The NCI expects that clinical trial data developed under a CTA or CRADA will be made available exclusively to Collaborator(s), and not to other parties.
4. When a Collaborator wishes to initiate a data request, the request should first be sent to the NCI, who will then notify the appropriate investigators (Group Chair for cooperative group studies, or PI for other studies) of Collaborator's wish to contact them.

5. Any data provided to Collaborator(s) must be in accordance with the guidelines and policies of the responsible Data Monitoring Committee (DMC), if there is a DMC for this clinical trial.
6. Any manuscripts reporting the results of this clinical trial should be provided to CTEP for immediate delivery to Collaborator(s) for advisory review and comment prior to submission for publication. Collaborator(s) will have 30 days from the date of receipt for review. An additional 30 days may be requested in order to ensure that confidential and proprietary data, in addition to Collaborator(s)'s intellectual property rights, are protected. Copies of abstracts should be provided to Collaborator(s) for courtesy review following submission, but prior to presentation at the meeting or publication in the proceedings. Copies of any manuscript and/or abstract should be sent to the following address and the Regulatory Affairs Branch will then distribute them to Collaborator(s):

   Regulatory Affairs Branch, CTEP, DCTDC, NCI
   9609 Medical Center Drive
   Bethesda, MD 20892
   email: info@ctep.nci.nih.gov

11 HUMAN SUBJECTS PROTECTIONS

11.1 RATIONALE FOR SUBJECT SELECTION

Non-Hodgkin's lymphomas affect all races and genders. However, males are more likely than females to be affected and this will be reflected in the gender distribution of our cases. We have selected mantle cell lymphomas for inclusion in this trial because they are considered to be incurable with chemotherapy. Thus, they would potentially benefit from the use of a novel agent like Bortezomib combined with a chemotherapy regimen like EPOCH-R. Additionally, the administration of maintenance Bortezomib therapy could result in an improved clinical outcome. Patients under the age of 18 are excluded because mantle cell lymphoma is rare in young patients, and the inclusion of an occasional younger patient will not provide generalizable information that would justify their inclusion on this phase II study. Additionally, patients with HIV infection will be excluded due to the severe immunosuppression of this therapy, and pregnant or nursing mothers are excluded because of the potential teratogenic effects of therapy.

11.2 PARTICIPATION OF CHILDREN

Subjects under the age of 18 are excluded because mantle cell lymphoma is rare in young patients; and the inclusion of an occasional younger patient will not provide generalizable information that would justify their inclusion on this study

11.3 PARTICIPATION OF SUBJECTS UNABLE TO GIVE CONSENT

Adults unable to give consent were excluded from enrolling in the protocol. However, re-consent may be necessary and there is a possibility, though unlikely, that subjects could become decisionally impaired. For this reason and because there is the prospect of direct benefit to subjects from treatment, including ongoing follow-up for detection of early relapse and to allow for the return of incidental findings of genetic testing (Sections **6.4.3** and **Error! Reference source not found.)** all subjects ≥ age 18 will be offered the opportunity to fill in their wishes for research and care, and assign a substitute decision maker on the "NIH Advance Directive for

Health Care and Medical Research Participation" form so that another person can make decisions about their medical care in the event that they become incapacitated or cognitively impaired during the course of the study.

Note: The PI or AI will contact the NIH Ability to Consent Assessment Team (ACAT) for evaluation to assess ongoing capacity of the subjects and to identify an LAR, as needed.

Please see section **11.5.1** for consent procedure.

11.4 EVALUATION OF BENEFITS AND RISKS/DISCOMFORTS

Patients may or may not obtain direct benefit from treatment with Bortezomib combined with EPOCH-R. Results from a phase I/II study of Bortezomib in combination with EPOCH shows the combination to have tolerable side effects.

11.4.1 Risks related to CT and PET scans

CT and PET scans often use a contrast agent. There is a small risk of having a reaction to the contrast and most often include nausea, pain in the vein where the contrast is given, headache, metallic and/ or bitter taste in the mouth and a warm, flushing feeling. Rarely, some people have more severe allergic reactions to the contrast which may include skins rashes, shortness of breath, wheezing or low blood pressure.

11.4.2 Risks from Radiation Exposure

The procedures for performing the CT scans will follow clinical policies, no special procedures apply to these additional assessments for research purposes. On this study patients who are given Parts A, B and Part C would have received additional scans during treatment; however, as all patients are in long-term follow-up on the study only (as of November 2021), the risks described in the protocol and consent will apply to the imaging for follow-up only. Accordingly: patients will receive up to up to one (1) CT scans of the chest, abdomen, and pelvis and two (2) [18F]-FDG PET scans as well undergo up to two (2) CT guided biopsies.

The total additional radiation dose for research purposes will be approximately 3.9 rem.

11.4.3 Risks from Blood Draws

The possible side effects of drawing blood include pain, bleeding, bruising, dizziness, light-headedness, fainting, and rarely a local blood clot formation or infection with redness and irritation of the vein.

11.4.4 Risks from Bone Marrow Aspiration/ Biopsy

Bone marrow biopsy is minimally invasive and is typically a very safe procedure. Usually, the hipbone is numbed with anesthesia. Using a needle, the solid and liquid portion of bone marrow is taken out. This procedure causes some pain. Very rarely, infection or bleeding may occur at the needle site.

11.4.5 Risks from Tumor Biopsy

The likely side effects include discomfort or pain, redness, swelling, and/or bruising at the site of the needle insertion. Bleeding from the site of the needle insertion is a less likely risk. Rarely, significant infection or bleeding from this procedure, allergic reaction to the anesthetic, or formation of a scar at the site of needle entry occurs.

11.4.6 Psychological or Social Risks Associated with Loss of Privacy

Learning of genetic risks for another disease or disability may be upsetting and cause distress.

11.4.7 Privacy Risks Associated with Return of Incidental or Secondary Findings

It may be possible that genetic information could be used to help identify the participant and/or participant's relatives.

11.4.8 Risks from Catheter Insertion

11.5 INSERTION OF CATHETERS CAN CAUSE, SITE PAIN, INFLAMMATION OF THE VEIN, BRUISING, INFECTION, BLOOD CLOTS, LEAK OF THE INFUSED LIQUID, AND IF THIS IS A CENTRAL LINE, PUNCTURE OF THE LUNG THAT CAN RESULT IN LUNG COLLAPSECONSENT PROCESS AND DOCUMENTATION

The informed consent document will be provided as a physical or electronic document to the participant or consent designee(s) as applicable for review prior to consenting. A designated study investigator will carefully explain the procedures and tests involved in this study, and the associated risks, discomforts and benefits. In order to minimize potential coercion, as much time as is needed to review the document will be given, including an opportunity to discuss it with friends, family members and/or other advisors, and to ask questions of any designated study investigator. A signed informed consent document will be obtained prior to entry onto the study.

The initial consent process as well as re-consent, when required, may take place in person or remotely (e.g., via telephone or other NIH approved remote platforms used in compliance with policy, including HRPP Policy 303) per discretion of the designated study investigator and with the agreement of the participant/consent designee(s). Whether in person or remote, the privacy of the subject will be maintained. Consenting investigators (and participant/consent designee, when in person) will be located in a private area (e.g., clinic consult room). When consent is conducted remotely, the participant/consent designee will be informed of the private nature of the discussion and will be encouraged to relocate to a more private setting if needed.

Consent will be documented with required signatures on the physical document (which includes the printout of an electronic document sent to participant) or as described below, with a manual (non-electronic) signature on the electronic document. When required, witness signature will be obtained similarly as described for the investigator and participant.

<u>Manual (non-electronic) signature on electronic document</u>:

When a manual signature on an electronic document is used for the documentation of consent at the NIH Clinical Center, this study will use the following to obtain the required signatures:

- Adobe platform (which is not 21 CFR Part 11 compliant); or,
- iMedConsent platform (which is 21 CFR Part 11 compliant)

During the consent process, participants and investigators will view individual copies of the approved consent document on screens at their respective locations (if remote consent); the same screen may be used when in the same location but is not required.

Both the investigator and the participant will sign the document using a finger, stylus or mouse.

Note: Refer to the CCR SOP PM-2, Obtaining and Documenting the Informed Consent Process for additional information (e.g., verification of participant identity when obtaining consent remotely) found here.

11.5.1 Consent Process for Adults Who Lack Capacity to Consent to Research Participation

For participants addressed in section **11.3**, an LAR will be identified consistent with Policy 403 and informed consent obtained from the LAR, as described in Section **11.5**.

12 REGULATORY AND OPERATIONAL CONSIDERATIONS

12.1 STUDY DISCONTINUATION AND CLOSURE

This study may be temporarily suspended or prematurely terminated if there is sufficient reasonable cause. Written notification, documenting the reason for study suspension or termination, will be provided by the suspending or terminating party to study participants, investigator, funding agency, and regulatory authorities. If the study is prematurely terminated or suspended, the Principal Investigator (PI) will promptly inform study participants, the Institutional Review Board (IRB), and sponsor and will provide the reason(s) for the termination or suspension. Study participants will be contacted, as applicable, and be informed of changes to study visit schedule.

Circumstances that may warrant termination or suspension include, but are not limited to:

- Determination of unexpected, significant, or unacceptable risk to participants
- Demonstration of efficacy that would warrant stopping
- Insufficient compliance to protocol requirements
- Data that are not sufficiently complete and/or evaluable
- Determination that the primary endpoint has been met
- Determination of futility

Study may resume once concerns about safety, protocol compliance, and data quality are addressed, and satisfy the sponsor, IRB and as applicable, Food and Drug Administration (FDA).

12.2 QUALITY ASSURANCE AND QUALITY CONTROL

The clinical site will perform internal quality management of study conduct, data and biological specimen collection, documentation and completion. An individualized quality management plan will be developed to describe a site's quality management.

Quality control (QC) procedures will be implemented beginning with the data entry system and data QC checks that will be run on the database will be generated. Any missing data or data anomalies will be communicated to the site for clarification/resolution.

Following written Standard Operating Procedures (SOPs), the monitors will verify that the clinical trial is conducted and data are generated and biological specimens are collected, documented (recorded), and reported in compliance with the protocol, International Conference on Harmonisation Good Clinical Practice (ICH GCP), and applicable regulatory requirements (e.g., Good Laboratory Practices (GLP), Good Manufacturing Practices (GMP)).

The investigational site will provide direct access to all trial related sites, source data/documents, and reports for the purpose of monitoring and auditing by the sponsor, and inspection by local and regulatory authorities.

12.3 CONFLICT OF INTEREST POLICY

The independence of this study from any actual or perceived influence, such as by the pharmaceutical industry, is critical. Therefore, any actual conflict of interest of persons who have a role in the design, conduct, analysis, publication, or any aspect of this trial will be disclosed and managed. Furthermore, persons who have a perceived conflict of interest will be required to have such conflicts managed in a way that is appropriate to their participation in the design and conduct of this trial. The study leadership in conjunction with the National Cancer Institute has established policies and procedures for all study group members to disclose all conflicts of interest and will establish a mechanism for the management of all reported dualities of interest.

12.4 CONFIDENTIALITY AND PRIVACY

Participant confidentiality and privacy is strictly held in trust by the participating investigators, their staff, and the sponsor(s). This confidentiality is extended to cover testing of biological samples and genetic tests in addition to the clinical information relating to participants. Therefore, the study protocol, documentation, data, and all other information generated will be held in strict confidence. No information concerning the study or the data will be released to any unauthorized third party without prior written approval of the sponsor.

All research activities will be conducted in as private a setting as possible.

The study monitor, other authorized representatives of the sponsor, representatives of the Institutional Review Board (IRB), and/or regulatory agencies may inspect all documents and records required to be maintained by the investigator, including but not limited to, medical records (office, clinic, or hospital) and pharmacy records for the participants in this study. The clinical study site will permit access to such records.

The study participant's contact information will be securely stored at the clinical site for internal use during the study. At the end of the study, all records will continue to be kept in a secure location for as long a period as dictated by the reviewing IRB, Institutional policies, or sponsor requirements.

Study participant research data, which is for purposes of statistical analysis and scientific reporting, will be transmitted to and stored at the NCI CCR. This will not include the participant's contact or identifying information. Rather, individual participants and their research data will be identified by a unique study identification number. The study data entry and study management systems used by the clinical site(s) and by NCI CCR research staff will be secured and password protected. At the end of the study, all study databases will be archived at the NIH.

To further protect the privacy of study participants, a Certificate of Confidentiality has been issued by the National Institutes of Health (NIH). This certificate protects identifiable research information from forced disclosure. It allows the investigator and others who have access to research records to refuse to disclose identifying information on research participation in any civil, criminal, administrative, legislative, or other proceeding, whether at the federal, state, or local level. By protecting researchers and institutions from being compelled to disclose

information that would identify research participants, Certificates of Confidentiality help achieve the research objectives and promote participation in studies by helping assure confidentiality and privacy to participants.

13 PHARMACEUTICAL INFORMATION

13.1 BORTEZOMIB

13.1.1 Supply

Bortezomib is being supplied by Millennium Pharmaceuticals, Inc. directly to the Clinical Center Pharmacy. Dr. Wilson, as sponsor of the study, will be responsible for complete study drug accountability in accordance with good clinical practices and ensuring that the study drug is labeled for clinical trial use only.

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

13.1.2 Storage and Stability

Vials containing lyophilized BORTEZOMIB for Injection should be stored according to the label requirements. For the United States, store at USP Controlled Room Temperature which is 25ºC (77ºF); storage temperature excursions are 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 the sponsor will notify the investigator should this information be revised during the conduct of the study.

13.1.3 Preparation and Administration

Drug will be administered only to eligible patients under the supervision of the investigator or identified subinvestigator(s). Patients may be treated on an outpatient basis, if possible.

The drug will be prepared under the supervision of a pharmacist, or appropriately qualified and trained personnel. 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 calculation. 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 within a cycle, as determined by an unscheduled weight assessment, then the patient's dose should be recalculated at that time based on clinical judgment.

The appropriate amount of bortezomib will be drawn from the injection vial and administered as an 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.

There must be at least 72 hours between each dose of bortezomib.

13.1.4 Product Destruction

For commercially-labeled bortezomib for IND-exempt studies, please contact your Millennium Clinical Operations representative to arrange for return of study drug procedures. Any unused or

expired bortezomib must be returned to Millennium. Be sure to document drug return on your drug accountability logs.

13.1.5 Potential Risks of Bortezomib

To date, more than 436,000 patients have been treated with bortezomib in both clinical trials investigating its use in hematological malignancies and solid tumors, and in patients who were treated with commercially available bortezomib.

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

The known anticipated risks of bortezomib therapy are presented in Table 11-1 and Table 11-2. These risks are grouped according to the combined frequency observed in an integrated analysis of AEs in sponsored clinical studies of single-agent bortezomib dosed at 1.3 mg/m2 twice weekly on a 21-day schedule, in patients with multiple myeloma and mantle cell lymphoma.

13.1.6 Precautions and Restrictions

It is not known what effects bortezomib 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 bortezomib, or agree to completely abstain from heterosexual intercourse.

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

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 (i.e., status post-vasectomy) 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 13-1 Known Anticipated Risks of BORTEZOMIB 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*, anemia*
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 hemorrhage
Gastrointestinal Disorders	
Most common	Constipation, diarrhea*, nausea, vomiting*
Very common	abdominal pain (excluding oral and throat)
Common	Dyspepsia, pharyngolaryngeal pain, gastroesophageal reflux, abdominal distension, gastritis, stomatitis, mouth ulceration, dysphagia, gastrointestinal hemorrhage*, lower gastrointestinal hemorrhage*± rectal hemorrhage
Uncommon	Eructation, gastrointestinal pain, tongue ulceration, retching, upper gastrointestinal hemorrhage*, hematemesis*, oral mucosal petechiae, ileus paralytic*, ileus, odynophagia, enteritis, colitis, esophagitis, enterocolitis, diarrhea hemorrhagic, acute pancreatitis*, intestinal obstruction
General Disorders and Administration Site Conditions	
Most common	Fatigue, pyrexia
Very common	Chills, edema 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	Hyperbilirubinemia, hepatitis*±
Immune System Disorders	
Uncommon	Drug hypersensitivity, angioedema
Infections and Infestations	

Table 13-1 Known Anticipated Risks of BORTEZOMIB by MedDRA System Organ Class, Observed Incidence, and Preferred Term	
System Organ Class **Observed Incidence**	**Preferred Term**
Very common	Upper respiratory tract infection, nasopharyngitis, pneumonia*, Herpes zoster*
Common	Lower respiratory tract infection*, sinusitis, pharyngitis, oral candidiasis, urinary tract infection*, sepsis∗, bacteremia*, 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 esophagitis±
Injury, Poisoning, and Procedural Complications	
Common	Fall
Uncommon	Subdural hematoma
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	Hyperglycemia, hypoglycemia, hyponatremia, hypokalemia, hypercalcemia*
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	Tumor 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
Uncommon	Convulsion, loss of consciousness, ageusia, encephalopathy, paralysis*,autonomic neuropathy, reversible posterior leukoencephalopathy syndrome±, posterior reversible encephalopathy syndrome ♦

Table 13-1 Known Anticipated Risks of BORTEZOMIB by MedDRA System Organ Class, Observed Incidence, and Preferred Term	
System Organ Class **Observed Incidence**	**Preferred Term**
Psychiatric Disorders	
Very common	Anxiety, insomnia
Common	Confusional state
Uncommon	Delirium
Renal and Urinary Disorders	
Common	Renal impairment*, renal failure*, hematuria
Uncommon	Micturition disorder
Respiratory, Thoracic, and Mediastinal Disorders	
Very common	Cough, dyspnea
Common	Epistaxis, dyspnea exertional, pleural effusion*, rhinorrhea, hypoxia*, pulmonary edema*
Uncommon	Hemoptysis*, acute respiratory distress syndrome*, respiratory failure*, pneumonitis*, lung infiltration, pulmonary alveolar hemorrhage*, 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 hemorrhage*
Source: VELCADE® Investigator's Brochure Edition 16. 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. ♦ Effective MedDRA update to version 14.0, the term 'reversible posterior leukoencephalopathy syndrome' updated to 'posterior reversible encephalopathy syndrome (PRES)'.	

Table 113-2 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
Skin and subcutaneous system disorders	

Table 113-2 Reports of Adverse Reactions From Postmarketing Experience	
System Organ Class Preferred Term	**Observed Incidence[a]**
Acute febrile neutrophilic dermatosis	Unknown
Toxic epidermal necrolysis	Unknown
Source: VELCADE® Investigator's Brochure Edition 16. 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 bortezomib include hepatic failure and QT prolongation. Fatal outcomes have been reported.
Women of childbearing potential should avoid becoming pregnant while being treated with bortezomib. 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 bortezomib may be found in the current Investigator's Brochure.

13.1.7 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 **8.5**).

Bortezomib should be given prior to the rituximab infusion.

13.2 RITUXIMAB

Refer to the FDA approved package insert for complete product information.

13.2.1 Supply

Commercially available in single-use vials containing 10 mL (100 mg) or 50 mL (500 mg) of rituximab solution at a concentration of 10 mg/mL.

13.2.2 Storage

Rituximab vials should be stored in a secure refrigerator at 2º to 8°C.

13.2.3 Preparation

Rituximab will be diluted to a final volume of 0.9% Sodium Chloride or 5% Dextrose Injection to prepare a standard product with concentration of 2 mg/ml. Caution should be taken during the preparation of the drug, as shaking can cause aggregation and precipitation of the antibody.

13.2.4 Stability

After dilution, rituximab is stable at 2-8 degrees C (36-46 degrees F) for 24 hours and for an additional 24 hours at room temperature.

13.2.5 Administration

A peripheral or central intravenous line will be established. During rituximab infusion, a patient's vital signs (blood pressure, pulse, respiration, temperature) should be monitored according to the standard of care. Medications readily available for the emergency management of anaphylactoid reactions should include: epinephrine (1:1000, 1 mg/mL) for subcutaneous injection, diphenhydramine hydrochloride for intravenous injection, and resuscitation equipment.

Prophylaxis against hypersensitivity and infusion-related reactions associated with rituximab will include acetaminophen 650 mg and diphenhydramine hydrochloride 50-100 mg administered 30 to 60 minutes prior to starting rituximab. Patients will also receive their first dose of prednisone 60 mg/m^2 (or a glucocorticoid equivalent dose of an alternative steroid) at least 60 minutes before rituximab treatment commences.

Rituximab will be administered as an intravenous infusion at 375 mg/m^2 on day 1 of each cycle of EPOCH after bortezomib (when both drugs are given on the same day) and before starting etoposide + doxorubicin + vincristine administration. Rituximab infusions will be administered to patients primarily in an outpatient clinic setting.

First dose:

The initial dose rate at the time of the first rituximab infusion should be 50mg/hour (25 mL/hr) for the first 30 minutes. If no toxicity is seen, the dose rate may be escalated gradually in 50 mg/hour (25 mL/h) increments at 30 minute intervals) to a maximum of 400 mg/hour (maximum rate = 200 mL/h).

Second and Subsequent Doses (select the appropriate administration timing):

90-minute Administration

If the first dose of rituximab was well tolerated, subsequent doses may be administered over 90 minutes with 20% of the total dose given in the first 30 minutes, and remaining 80% of the total dose administered over the subsequent 60 minutes; e.g.:

Two-Step Rate Escalation	Volume to administer (X mL)

1st portion (0 – 30 minutes)	$\frac{\text{Total Dose (mg)}}{2} \times 0.2 = \text{X mL (over 30 min)}$
2nd portion (30 – 90 minutes)	$\frac{\text{Total Dose (mg)}}{2} \times 0.8 = \text{X mL (over 60 min)}$

Special Note: The 90-minute infusion scheme is not recommended for patients with clinically significant cardiovascular disease or high circulating lymphocyte counts (≧5000/mcL).

Standard Administration for Second & Subsequent Infusions

Patients who tolerate initial treatment without experiencing infusion-related adverse effects but for whom the 90-minute infusion scheme during subsequent treatments is considered inappropriate, may receive subsequent rituximab doses at the Standard Rate for Subsequent Infusions, which is as follows:

Begin at an initial rate of 100 mg/hour (50 mL/h) for 30 minutes. If administration is well tolerated, the administration rate may be escalated gradually in 100-mg/hour (50-mL/h) increments at 30-minute intervals to a maximum rate of 400 mg/hour (maximum rate = 200 mL/h).

CAUTION: DO NOT ADMINISTER RITUXIMAB AS AN INTRAVENOUS PUSH OR BOLUS.

13.2.6 Toxicities

Common toxicities include fever, chills, nausea, asthenia, headache, angioedema, pruritis and rash. Leukopenia occurs in approximately 10% but grade 3 or 4 neutropenia is uncommon. Hypotension occurred in 10% of patients during rituximab infusion, and serious bronchospasm and urticaria associated with rituximab infusion each occurred in fewer than 10% of patients. Less common toxicities include abdominal pain, vomiting, thrombocytopenia, anemia, myalgia, arthralgia, dizziness, and rhinitis. Recently, in patients receiving Rituximab, there have been reports of hepatitis B virus reactivation with fulminant hepatitis, hepatic failure and death in some patients with hematological malignancies.

13.3 CYCLOPHOSPHAMIDE

13.3.1 Supply

Commercially available as a lyophilized powder for reconstitution in vials containing 100 mg, 200 mg, 500 mg, 1gm, and 2 gm of cyclophosphamide.

13.3.2 Storage and preparation

Intact vials should be stored at room temperature (not to exceed 30ºC). Reconstitute with appropriate amounts of 0.9% NaCl to produce a final concentration of 20 mg/ml. Discard solution after 24 hours at room temperature. Stable up to 6 days if refrigerated (2º-8ºC).

13.3.3 Administration

Cyclophosphamide will be diluted in D5W or 0.9% NaCl and administered as an intravenous infusion over 30minutes. Patients will be instructed to drink an adequate amount of fluids and empty their bladders frequently during cyclophosphamide administration.

13.3.4 Hydration Guidelines

All patients should receive 0.9%NS at the following volumes (based on cyclophosphamide dose levels) and rates with half the specified volume given before starting cyclophosphamide administration and half the volume given after completing the cyclophosphamide administration.

Cyclophosphamide Dosage Levels	Fluid Volume and Administration Rate
1 & 2	1000 mL 0.9%NS @ 300 – 500 mL/h
Levels 3, 4, & 5	2000 mL 0.9%NS @ 300 – 500 mL/h
Levels ≥6	2500 mL 0.9%NS @ 300 – 500 mL/h

13.4 DOXORUBICIN

Refer to the FDA approved package insert for complete product information.

13.4.1 Supply

Commercially available as a lyophilized powder for reconstitution in 10, 20, 50, and 100 mg vials. Also available as 2 mg/ mL solution for injection in 10, 20, 50, and 200 mg vials.

13.4.2 Preparation and stability

Intact vials of doxorubicin solution for injection should be stored in the refrigerator (2º-8ºC). Intact vials of doxorubicin lyophilized powder for reconstitution should be stored at room temperature (not to exceed 30ºC).

Reconstitute vials of doxorubicin powder with appropriate amounts of 0.9% NaCl to produce a final concentration of 2 mg/ml. Reconstituted solutions are stable for 7 days at room temperature and 15 days under refrigeration when protected from light.

13.4.3 Toxicities

Myelosuppression, stomatitis, alopecia, nausea and vomiting, and acute and chronic cardiac toxicity, manifested as arrhythmias or a congestive cardiomyopathy, the latter uncommon at total cumulative doses less than 500 mg/m^2. The drug causes local necrosis if infiltrated into subcutaneous tissue. Please refer to the package insert for a complete listing of all toxicities.

13.5 VINCRISTINE

Refer to the FDA approved package insert for complete product information.

13.5.1 Supply

Commercially available as a 1 mg/mL solution for injection in 1 mg, 2 mg, and 5 mg vials.

13.5.2 Stability

Vials should be stored at 2º-8ºC and should be protected from light.

13.5.3 Toxicities

Peripheral neuropathy, autonomic neuropathy, alopecia. Local necrosis if injected subcutaneously. Please refer to the package insert for a complete listing of all toxicities.

13.6 ETOPOSIDE

13.6.1 Supply

Commercially available as a concentrate for parenteral use in 100 mg vials; each ml contains 20 mg etoposide, 2 mg citric acid, 30 mg benzyl alcohol, 80 mg polysorbate 80, 650 mg of polyethylene glycol 300, and 30.5% alcohol.

13.6.2 Toxicities

Myelosuppression, nausea, vomiting, anaphylactoid reactions, alopecia, and hypotension if infusion is too rapid. Please refer to the package insert for a complete listing of all toxicities.

13.7 ADMINISTRATION OF VINCRISTINE/DOXORUBICIN/ETOPOSIDE

Stability studies conducted by the Pharmaceutical Development Section, Pharmacy Department, NIH Clinical Center, have demonstrated that admixtures of vincristine, doxorubicin, and etoposide in 0.9% Sodium Chloride Injection, USP (0.9%NS) at concentrations, respectively, of 1, 25, and 125 mcg/mL; 1.4, 35, and 175 mcg/mL; 2, 50, and 250 mcg/mL; and 2.8, 70, 350 mcg/mL are stable for at least 36 hours at room temperature when protected from light. Also, admixtures containing vincristine, doxorubicin, and etoposide concentrations of 1.6, 40, and 200 mcg/mL are stable for at least 30 hours at 32°C.

For this study, etoposide, doxorubicin, and vincristine comprising a daily dose (a 24-hour supply) will be diluted in 0.9%NS. Product containers will be replaced every 24 hours to complete the planned duration of infusional treatment. Product volumes will be determined by the amount of etoposide present in a 24-hour supply of medication. For daily etoposide doses ≤130 mg, admixtures will be diluted in approximately 500 mL 0.9%NS. For daily etoposide doses >130 mg, admixtures will be diluted in approximately 1000 mL 0.9%NS.

Etoposide + doxorubicin + vincristine admixtures will be administered by continuous IV infusion over 96 hours with a suitable rate controller pump via a central venous access device.

Temporary PICC lines or permanent lines may be used. Extravasation of these diluted agents has not caused local tissue damage due to their low concentrations in the solution.

13.8 PREDNISONE

13.8.1 Supply

Commercially available in a large number of oral dosage strengths including pills and liquid formulations.

13.8.2 Storage

Tablets should be stored in well-closed containers at temperatures between 15-30^{o}C.

13.8.3 Administration

Prednisone utilization will be simplified by using only 20- and 50-mg tablets to produce individual doses and by stratifying prednisone doses by a patient's body surface area (BSA), according to the chart below. These are recommendations and not requirements.

BSA (m^2)	Each Dose
1.25 – 1.49	80 mg

1.5 – 1.83	100 mg
1.84 – 2.16	120 mg
2.17 – 2.41	140 mg
2.42 – 2.6	150 mg
2.61-2.69	160 mg
2.7 – 3	170 mg

13.8.4 Toxicities

Proximal muscle weakness, glucose intolerance, thinning of skin, redistribution of body fat, Cushingoid facies, immunosuppression, propensity to gastrointestinal ulceration. Please refer to the package insert for a complete listing of all toxicities.

13.9 FILGRASTIM (G-CSF/NEUPOGEN®)

Refer to the FDA approved package insert for complete product information.

13.9.1 Supply

Commercially available in single use vials containing 300 mcg (1 mL/vial) and 480 mcg/vial (300 mcg/ml, 1.6 mlvial).

13.9.2 Storage

Should be stored at 2º-8ºC (do not freeze and do not shake) and is stable for at least 1 year at this temperature.

13.9.3 Administration

Filgrastim will be given by subcutaneous injection; patient or other caregiver will be instructed on proper injection technique.

10.8.2 Toxicities

Rare anaphylactic reactions with the first dose; bone pain at sites of active marrow with continued administration. Local reactions at injection sites. Constitutional symptoms, increased alkaline phosphatase, LDH, uric acid; worsening of pre-existing inflammatory conditions. Please refer to the package insert for a complete listing of all toxicities.

14 ADMINISTRATIVE REQUIREMENTS

14.1 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.

14.2 ETHICAL CONSIDERATIONS

The study will be conducted in accordance with ethical principles founded in the Declaration of Helsinki. 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), safety updates, annual progress reports, and any revisions to these documents will be provided to the IRB/IEC by the investigator. Informed consent documents will be provided to Millennium for their review.

14.3 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).

14.4 PATIENT CONFIDENTIALITY

In order to maintain patient privacy, all data capture records, drug accountability records, study reports and communications will identify the patient by name and/or 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.

14.5 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. The investigator will submit all protocol modifications to Millennium and the regulatory authority(ies) in accordance with the governing regulations.

14.6 ON-SITE AUDITS

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.

14.7 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 bortezomib will be treated and disposed of as hazardous waste in accordance with governing regulations.

14.8 RECORD RETENTION

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

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16 APPENDIX A: PROCEDURE FOR SERUM SEPARATION

This procedure is for use with blood tubes or syringes containing no anticoagulant. In some cases the blood will be received in vacutainer tubes containing a serum separator. Use of these serum separator tubes (SST) simplifies the recovery of serum after centrifugation since the gel separator will be found between the cell layer and the serum.

Complete all required data entry. Prepare transmittal form to accompany sample. Assign each sample a unique Sample Accession Number. Contact the Clinical Support Laboratory at (301) 846-5125 to receive one or more Sample Accession Numbers for use. Laboratory staff can also be contacted by email:

To: Theresa Burks – burkst@mail.nih.gov
CC: Helen Rager (Supervisor) – ragerh@mail.nih.gov
CC: Dr. Mingzhu Zhu (Lab Head) – zhum@mail.nih.gov

The sample should be allowed to clot at room temperature for approximately 30 minutes. If possible the sample should be processed immediately after clotting. If it is not possible to proceed immediately the sample should be refrigerated or placed in a sealed bag on wet ice until processing can be performed. Both the draw time and time when processing was completed should be recorded.

Set the centrifuge temperature to 4°C. Centrifuge the sample for 10 minutes at 2000 rpm (approx. 1170 x g) in the RC-3B centrifuge with no brake (2300 rpm Sorvall RT6000B). The centrifugation time does not include the time required for the rotor to reach the target speed. To determine centrifugation speed for different centrifuge/rotor combinations see the centrifuge manual or visit the following website:
http://researchlink.labvelocity.com/tools/conversionTools/CentrifugationTool.jhtml

Prior to initiating sample processing or during the centrifugation step label 2 ml Nunc cryovials (NUNC 368632 1.8 ml round bottom cryovial or equivalent) with the sample Accession Number. If multiple blood tubes have been received for a single patient/timepoint also label a 15 or 50 ml polypropylene tube depending on expected recovery volume.

Carefully remove the serum layer (*the translucent first layer of fluid)* without disturbing the clot. Do not attempt to recover serum from the side of the retracted clot as doing so will result in contamination of the serum. If it is noted that the serum has not been cleanly removed transfer the recovered serum to a 15 ml polypropylene tube and repeat centrifugation.

If only a single tube of blood was received for serum recovery aliquot directly to the labeled Nunc cryovials at 1ml per vial (1 10ml tiger top (SST) or red top vacutainer = approximately 4 vials, 2 tubes = >6 vials).

If multiple blood tubes are received for a single patient/timepoint, transfer the serum from each tube into a single 15 or 50 ml centrifuge tube. Mix the contents by repeated pipetting or by replacing the lid and inverting the tube at least 6 times prior to aliquoting.

Transfer filled vials to the -70°C freezer. If the laboratory does not have access to a -70°C freezer (or colder storage such as vapor phase LN2) the serum should be frozen solid on dry ice before transfer to the -20°C freezer. If samples are held at temperatures outside the range of –60 to -86°C please note the storage temperature on the transmittal form.

Include any comments concerning adequacy of the specimen (e.g., gross hemolysis) on the transmittal form.

17 APPENDIX B: EPOCH ADMIXTURES: PREPARATION AND ADMINISTRATION

Preparation

All 3-in-1 admixtures dispensed from the Pharmacy will contain a 24-hour supply of etoposide, doxorubicin, and vincristine, *PLUS* 40 mL overfill (excess) fluid and a proportional amount of drug to compensate for volume lost in parenteral product containers and administration set tubing.

Etoposide Dose	**Volume of Fluid Containing a Daily Dose**	**Volume of Overfill** (fluid + drug)	**Total Volume in the Product** (including overfill)
≤ 130 mg	528 mL	40 mL	568 mL
> 130 mg	1056 mL	40 mL	1096 mL

Before dispensing 3-in-1 admixtures, Pharmacy staff will:

[1] Purge all air from the drug product container,
[2] Attach an administration set appropriate for use with a portable pump,
[3] The set will be primed close to its distal tip, and
[4] The set will be capped with a Luer-locking cap.

Pre-printed product labeling will identify the 'Total Volume To Infuse' and the 'Volume of Overfill (fluid + drug)'.

Bags will be exchanged daily for four consecutive days to complete a 96-hour drug infusion (unless treatment is interrupted or discontinued due to un-anticipated events).

Administration

Portable pumps used to administer etoposide + doxorubicin + vincristine admixtures will be programmed to deliver one of two fixed volumes at one of two corresponding fixed rates based on the amount of etoposide and fluid that is ordered (see the table, below).

Etoposide Dose	**Total Volume to Infuse per 24 hours**	**Volume of Overfill** (drug-containing fluid)*	**Administration Rate**
≤ 130 mg	528 mL	40 mL	22 mL/hour
> 130 mg	1056 mL	40 mL	44 mL/hour

*DO NOT attempt to infuse the overfill.

At the end of an infusion, some residual fluid is expected because overfill (excess fluid and drug) was added; however, nurses are asked to return to the Pharmacy for measurement any drug containers that appear to contain a greater amount of residual drug than expected.

Example at right: The amount of fluid remaining in a bag after completing a 24-hour infusion (1056 mL delivered).

18 APPENDIX C: PREGNANCY REPORTING FORM

Pregnancy Form

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: ___/___/_____ (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: _____

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: _____ • Normal live birth: _____
- Therapeutic abortion: _____ • Children born with defects: ____
- Elective abortion: _____ • Stillbirth: _____
- Other: _____ • 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

e-Pregnancy Form (27 November 2013)

MILLENNIUM
THE TAKEDA ONCOLOGY COMPANY

Pregnancy Form

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: _____

e-Pregnancy Form (27 November 2013)