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Institutional Review Board

Approval Notice

This institution has an approved assurance of compliance on file with HHS which covers this activity FWA 00006731 Federal Wide Assurance identification number

August 23, 2017

Francisco Hernandez-Ilizaliturri, MD

Francisco.Hernandez@RoswellPark.org

Dear Dr. Francisco Hernandez-Ilizaliturri:

On 8/22/2017, the IRB reviewed the following submission:

Type of Submission:	Modification and Continuing Review
Type of Review:	☐ Full Board ☒ Expedited ☐ Exempt ☐ Non-Human Research
Title of Study:	Phase II Clinical Trial of Rituximab in Combination with Pegfilgrastim in Patients with Indolent B-Cell (CD-20-Positive) Lymphoma
Investigator:	Francisco Hernandez-Ilizaliturri, MD
IRB ID:	MODCR00000440 / I 83106
Funding:	Name: ROSWELL PARK CANCER INSTITUTE
Grant ID:	None
IND, IDE, or HDE:	IND #I00396, HOLDER Roswell Park Cancer Institute
Documents Reviewed:	• I 83106 PROT AMD#7 CLN 10.4.13.pdf, Category: IRB Protocol;

The IRB approved the study from 8/22/2017 to 9/6/2018 inclusive. Before 9/6/2018 or within 30 days of study closure, whichever is earlier, you are to submit a continuing review with required explanations. You can submit a continuing review by navigating to the active study and clicking Create Modification / CR. If continuing review approval is not granted on or before 9/6/2018, approval of this study expires after that date.

The principal investigator is responsible for ensuring that the research complies with all applicable regulations. Any modifications in the research project are subject to approval by the Board prior to initiation by the investigator. The Board reserves the right to stop the research for violations of regulatory or IRB requirements.

A progress report must be submitted to the IRB at least one month prior to the expiration date noted above for continuing review as required by federal regulations and/or institutional requirements.

Please be advised that your research study may be audited periodically by the IRB for compliance.

This activity has been reviewed and approved by an IRB in accordance with the requirements of 45 CFR 46, including its relevant Subparts. This protocol fulfills, when

applicable, requirements for certifying FDA status for each investigational new drug or device.

The study documents have been submitted to Clinical Research Services (CRS) Compliance Office for processing prior to release and protocol implementation. Please contact CRS Compliance for information regarding the protocol implementation release date.

In conducting this protocol you are required to follow the requirements listed in the INVESTIGATOR MANUAL (HRP-103), including the reporting of Unanticipated Problems and any other Reportable New Information.

Sincerely,
Donald Handley MSc, MBA
Camille P Wicher, PhD, Esq., RN, MSN

RPCI PROTOCOL NO.: I 83106

PROTOCOL TITLE:

PHASE II CLINICAL TRIAL OF RITUXIMAB IN COMBINATION WITH PEGFILGRASTIM IN PATIENTS WITH INDOLENT B-CELL (CD-20-POSITIVE) LYMPHOMA.

PRESENTED TO AMGEN, INC.

ROSWELL PARK CANCER INSTITUTE
ELM AND CARLTON STREETS
BUFFALO, NY 14263

PRINCIPAL INVESTIGATOR:
FRANCISCO J. HERNANDEZ-ILIZALITURRI MD
TELEPHONE: 716 845-3560

This study is classified as a Roswell Park Cancer Institute Investigator Initiated Study with sponsorship from Amgen, Inc. group of companies

INITIAL SUBMISSION:	**DATE: JULY 11, 2006**
AMENDMENT #1	**DATE: OCTOBER 27, 2006**
AMENDMENT #2	**DATE: JULY 14, 2008**
AMENDMENT #3	**DATE: FEBRUARY 23, 2009**
AMENDMENT #4	**DATE: JANUARY 11, 2010**
AMENDMENT #5	**DATE: JULY 20, 2011**
AMENDMENT #6	**DATE: APRIL 16, 2012**
AMENDMENT #7	**DATE: OCTOBER 4, 2013**

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1. INTRODUCTION

Low grade and/or follicular non-Hodgkin's lymphoma

1.1 Background and Overview

Non-Hodgkin's lymphomas (NHLs) are a heterogeneous group of lymphoproliferative malignancies with variable patterns of behavior and responses to treatment [1]. Like Hodgkin's disease, NHL usually originates in lymphoid tissues and can spread to other organs. However, NHL is much less predictable than Hodgkin's disease and has a far greater predilection to disseminate to extranodal sites. The prognosis depends on the histologic type, stage, and treatment. The working formulation divides NHL into three prognostic groups: low-grade, intermediate-grade, and high-grade. Low-grade NHL subtypes have a median survival of approximately 6.2 years and although initially chemosensitive become increasingly more treatment-resistant as demonstrated by decreasing remission durations seen with subsequent therapeutic intervention. The vast majority of low-grade B-cell lymphomas are felt to be incurable with standard chemotherapy [2]. Therefore, new agents and treatment strategies need to be evaluated in these patients.

State-of-the-art treatment for non-Hodgkin's lymphoma (NHL) depends on the histologic type and stage. Recently, improvements in disease-free survival have been demonstrated in clinical trials (experimental therapy) that have improved on the best available accepted therapy (conventional or standard therapy) by combining them with novel target-specific agents (e.g. monoclonal antibodies [mAbs], etc.) Monoclonal antibodies (mAbs) were developed to target and kill tumors with less toxic effects on normal tissues. MAbs directed against tumor-associated antigens, offer less non-specific toxicity than most chemotherapy agents. One characteristic of monoclonal antibodies, is their ability to fix human complement and activate complement-mediated cytotoxicity (CMC). Another mechanism of anti-tumor activity by mAbs is antibody-dependent cellular cytotoxicity (ADCC) through the binding of the Fc portion of the monoclonal antibodies to Fc receptors on lymphocytes, monocytes, macrophages, granulocytes, and eosinophils. Among human antibodies, IgG1 is the only class that binds to macrophage Fc receptors. Clinical trials have evaluated a number of mAbs as potential treatments of hematological malignancies. The results of these clinical trials have been highly variable [3]. Nevertheless, the results of some clinical trials of mAbs have shown promising activity and safety in patients with B-cell lymphomas that were (at times) resistant or refractory to conventional treatments.

Rituximab is the first mAb approved by the US FDA for the treatment of cancer. It is a genetically engineered chimeric (human/murine) mAb with a human IgG1 constant region directed against the CD20 antigen found on the surface of normal and malignant B cells. A Phase III pivotal study demonstrated a 48% (80/166)

overall response rate in previously treated low grade and follicular B-cell lymphoma patients receiving out-patient rituximab at a dose of 375 mg/m^2/week for 4 weeks [4]. The median time to progression (TTP) was 13.1 months in responders. The most common side effect seen was an infusion-related symptom complex consisting of fever and chills/rigors which occurred in the majority of patients during the first antibody infusion. Rituximab has been combined with standard dose CHOP chemotherapy in a separate Phase II chemoimmunotherapy study in low grade NHL and demonstrated a 100% response rate with minimal additional toxicity than that typically seen with CHOP chemotherapy alone [5]. Furthermore, CHOP plus rituximab (CHOP-R) combination therapy has been evaluated in additional Phase II [6] and Phase III [7] studies. In the Phase III study a statistically significant improvement in complete response (CR) rate, disease-free survival (DFS) and overall survival (OS) was found in elderly patients with diffuse large B-cell lymphoma (DLBCL) treated with CHOP-R, compared to those treated with CHOP alone. Due to its unique immunologic mechanism(s) of action and good safety profile, rituximab has been studied in combination with other chemotherapeutic and/or biologic agents in an attempt to improve anti-tumor activity while decreasing the non-specific toxicities typically associated with a CHOP-like regimen alone. This current proposal plans to evaluate the safety and efficacy of a combination of rituximab plus Pegfilgrastim.

Modulating the innate immune system is an attractive strategy to improve rituximab activity. It has been demonstrated by various groups of investigators not only that neutrophils play a significant role in rituximab activity but that priming neutrophils with cytokines augments rituximab anti-tumor activity in pre-clinical models. Clinical studies using G-CSF in combination with rituximab had shown improvement in complete response rates when compared to rituximab monotherapy historical controls. As pegfilgrastim possesses different pharmacokinetics and pharmacodynamics than G-CSF, the biological interaction with rituximab might be stronger.

1.2 Rituximab Background

Rituximab is a chimeric monoclonal antibody, with mouse variable and human IgG1 constant regions that recognizes the CD20 antigen expressed on normal B cells and most malignant B-cell lymphomas. This antigen, important in cell cycle initiation and differentiation, is expressed strongly in over 90% of B-cell lymphomas [8]. Rituximab demonstrates specificity for CD20 and binds with an apparent affinity of 5.2 x 10^{-9}M. *In vitro* mechanism of action studies have demonstrated that this antibody binds human complement and lyses lymphoid B cell lines and it has significant activity in assays for antibody dependent cellular cytotoxicity. Rituximab has also been shown to have antiproliferative effects in titrated Thymidine incorporation assays and to directly induce apoptosis in several CD20-positive cell lines, while some other anti- CD20 antibodies do not [9]. High dose safety studies in cynomolgous monkeys have revealed no adverse clinical events. There were no significant abnormalities on laboratory tests or on

histopathological analysis. As predicted, the biologic effect of rituximab is manifested by B-cell depletion in peripheral blood (PB), lymph nodes (LN) and bone marrow (BM). Three weeks after 4 weekly doses there was a > 85% depletion of B cells in the lymph nodes and a > 75% decrease of B cells in the bone marrow. Recovery of the B cells in the peripheral blood (to > 75% of baseline) usually occurred within 60 days following the last dose.

1.2.1 Clinical Experience

Initial human clinical trials were conducted primarily in patients with multiple relapses of low-grade non-Hodgkin's lymphomas. A Phase I, single-dose, dose-escalation trial demonstrated that rituximab was well tolerated at doses ranging from 10 to 500 mg/m^2. The mean half-life ($t_{1/2}$) observed for rituximab in this study was 118 hours. CD20+ B-cells were rapidly depleted 24-72 hours following rituximab administration and remained depleted for 2-3 months [10]. A subsequent Phase I/II, multiple-dose study demonstrated that a regimen of four weekly IV infusions of rituximab at doses of 125-375 mg/m^2 was equally well tolerated. A total of 37 patients received four doses of 375 mg/m^2. The overall response rate in this study was 50%, with a mean time to progression of 10.2 months [11].

Adverse events reported in these studies have occurred largely with the first infusion. Fever, chills, headache, nausea, vomiting, rhinitis, and hypotension, generally of Grade 1 and 2 severities, have been reported. These symptoms have typically responded to an interruption of the antibody infusion with subsequent resumption at a slower infusion rate. Other adverse events have included transient neutropenia, thrombocytopenia, and asthenia.

A large Phase III study of four weekly infusions of rituximab at 375 mg/m^2 in patients with low-grade lymphoma has been completed. In this open-label, single arm, pivotal trial, 166 patients received weekly x four infusions of 375 mg/m^2 of rituximab. Adverse events (AE) were primarily related to the first infusion. There was a marked reduction in the incidence of AEs in subsequent infusions. (See Table 1 in the Rituximab Full Prescribing Information Booklet included in Appendix A for a list of possible adverse events). None of the patients in this trial developed a significant human anti-chimeric antibody response (HACA) [4].

Ninety-one percent of patients (151 of 166) were evaluable for efficacy and an overall response rate of 48% with 6% CR and 42% PR was achieved. The onset of response was documented as early as seven weeks and responses were seen in patients with bulky and extra nodal disease and in patients who had progressed following ABMT or anthracycline therapy. CT scans of responders were reviewed and

confirmed (blinded audit) by an independent panel of lymphoma experts following established response criteria. Complete response required all lymph nodes to regress to ≤ 1 x 1 cm and all signs and symptoms of disease to disappear. Median time to progression for responders was subsequently reached at 13.1 months.

Various schedules of rituximab for initial therapy of advanced stage follicular lymphoma have been completed and, in general, demonstrate higher overall response and CR rates (range of ~70% ORR, 38 % CR) compared to patients receiving rituximab following prior chemotherapy.

An open-label, single-arm, Phase II study of six infusions of rituximab (375 mg/m^2) interspersed among six cycles of CHOP chemotherapy has also been conducted in patients with low-grade lymphoma. The overall response rate in the 35 evaluable patients who completed all of the planned treatment was 100%, with a CR rate of 63%. Seven of 8 patients noted to be positive for the bcl-2 marker by polymerase chain reaction (PCR) converted to bcl-2 negative in bone marrow and/or blood following treatment suggesting the clearance of minimal residual disease in these patients. No significant additive toxicity was observed with the combination treatment [5]. Separate Phase II and Phase III studies of CHOP plus rituximab in intermediate-grade lymphoma have also demonstrated augmented anti-tumor activity without significant increases in toxicity [6, 7].

Rituximab has been shown to be active in the treatment of CD20-positive indolent and aggressive lymphomas and has been noted to have a favorable toxicity profile both as a single agent and in combination with CHOP and other chemotherapy regimens. The unique mechanism of action of rituximab supports its potential in augmenting anti-tumor activity when combined with other agents.

1.2.2. Cellular mediated immunity and Rituximab anti-tumor activity.

Despite its evident clinical efficacy, some patients develop disease progression while on therapy with rituximab or relapse after initial response. Up to 50% of indolent NHL patients who have received prior chemotherapy treated with single agent rituximab fail to demonstrate an objective anti-tumor response (i.e. PR or CR). Several mechanisms for tumor resistance to monoclonal antibody therapy had been formulated. NHL related factors postulated are CD20-antigen density expression, complement inhibitory protein expression (e.g. CD55, CD54) by NHL cells and tumor burden. On the other hand, host-related factors such as pharmacokinetics and pharmacogenomics may play a significant role in patients that do not respond to rituximab.

Several biological effects have been postulated as rituximab's primary mechanism of anti-tumor activity [12-22]. Major areas of interest are the activation of the innate immune system and/or induction of apoptosis following rituximab therapy [12-21]. Recently published *in vivo* studies demonstrate that FcγR receptor expression is necessary to eradicate NHL in a murine animal model, suggesting that antibody-dependent cellular cytotoxicity (ADCC) plays a significant role in rituximab's activity [21]. Furthermore, certain polymorphisms in the FcγRIIIa gene have been associated with variable clinical and molecular response to anti-CD20 mAb therapy in patients with indolent NHL [22].

FcR receptors mediate many of the cell-dependent functions of antibodies, including phagocytosis of antibody-bound antigens, activation of mast cells, complement activation and targeting/activation of natural killer (NK) cells. There are three types of Fc∂R receptors and eight subtypes. FcγRI or CD64 (high affinity) mediates phagocytosis by macrophages and neutrophils. FcγRIIb or CD32 (low affinity) that transduces inhibitory signals in B cells and finally FcγRIIIa (CD16), another low affinity receptor mediates the activation of natural killer cells to induce ADCC.

Based on prior studies, some investigators postulate that NK cells and macrophages mediate the destruction of NHL cells by rituximab; primarily via antibody dependent cellular cytotoxicity. The effects of neutrophils in tumor immunology may be largely underestimated. There is emerging evidence that polymorphonuclear cells (PMNs) are capable of not only migrating and infiltrating cancerous tissues but also in inducing anti-tumor activity.

We have demonstrated that neutrophil function is necessary for the anti-tumor activity of rituximab in a lymphoma mouse model [23]. Two sets of experiments were performed. Initially, NK cells were depleted in all mice using an anti-IL-2 receptor antibody (SCID mice lack T and B lymphocytes) to ensure tumor engraftment. Neutrophils were depleted in 2 groups of mice through serial intraperitoneal injections of a rat-mAb against Ly-6G, a 21-25kDa GIP-anchored protein expressed specifically on granulocytes (myeloid differentiation Gr-1 antigen). Tumor growth was similar regardless of the presence or absence of circulating neutrophils in control mice (data not shown). Among rituximab-treated animals, anti-Gr-1 antibody treatment eliminated the anti-tumor effect observed with rituximab (Figure. 1A).

To determine the contribution of NK cells to rituximab anti-tumor activity in NHL-bearing SCID mice we conducted a second series of experiments. NK cells were not depleted. All animals were inoculated with Raji cells via tail vein injection and half of the SCID mice underwent neutrophil

depletion as described above. Subsequently, animals were randomized to receive rituximab or placebo. Again, rituximab-treated animals with intact NK cells had better survival than untreated animals with or without NK cells. Moreover, SCID mice with intact neutrophil and NK function had the best response to treatment with rituximab (Figure. 1B).

The results from these experiments demonstrated that effector cells are indispensable for the biological activity of rituximab. In order to exclude any potential anti-tumor activity that could be contributed by rituximab-mediated CMC and to further validate this hypothesis, we investigated whether rituximab could activate the murine complement system and lyse lymphoma cells *in vitro*. We conducted standard ^{51}Cr release assays in the human lymphoma cell lines DHL-4 using rituximab or trastuzumab (isotype control) with sera isolated from human versus SCID or BALB-c mice as the source of complement proteins (final dilution 1:8). In the DHL-4 cell lines therapy with anti-CD20 mAb was ineffective in inducing CMC in the presence of either SCID or BALBc (immunocompetent) mouse serum. Rituximab-induced CMC was only seen in the presence of human serum (60% of cell lysis).

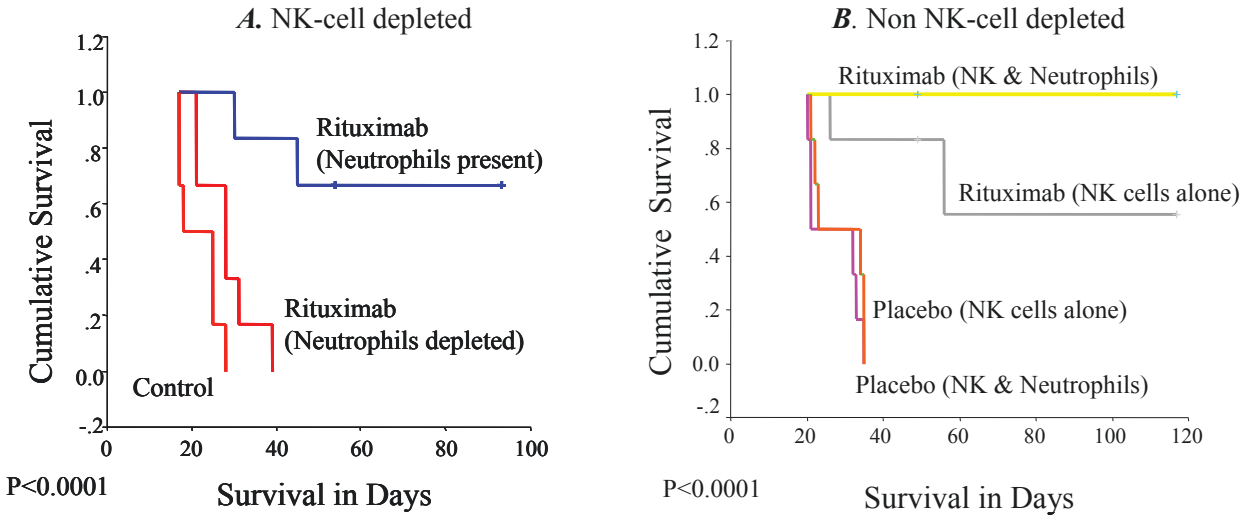

Figure. 1 Kaplan Meier Survival curves demonstrate that neutrophil depletion dramatically reduces the efficacy of Rituximab in the NK cell depleted (A) and in the non-NK cell depleted SCID mouse models (B).

1.3 Neutrophils and tumor immunology:

PMNs induce tumor destruction by several mechanisms. Tumor recruited neutrophils produce several cytotoxic mediators such as reactive oxygen species, proteases, membrane-perforating agents and soluble mediators of cell killing (TNFα, IL-β1 and INFs). A second mechanism of neutrophil-mediated anti-tumor

activity is by ADCC. PMNs express several subtypes of FcR receptors capable of inducing antibody-dependent cellular cytotoxicity such as FcRγIIa FcRγIIIa and FcRγIIIb.

Recruitment of neutrophils into the tumor bed is a process that requires a series of coordinated interactions between PMNs and endothelial cells. During this process, PMNs undergo different phenotypical changes that result in an activated effector cell. It is well known that the expression of P-, E- and L-selectin (CD62) and other adhesion molecules such as IMAC-1 (CD11b/CD18) play an important role in neutrophil trafficking. The interaction between neutrophil and other immune-effector cells activation and rituximab against B-cell malignancies and its relevance in the therapeutic strategies of NHL have not been extensively studied in the past.

1.4 Rationale for Rituximab and Pegfilgrastim:

We recently have proved that, host immune system activation by target-specific molecules such as G-CSF/GM-CSF enhances the biological activity of rituximab and potentially other mAbs [24]. To further explore the stimulation of effector cells as a strategy to enhance the biological activity of rituximab, we subsequently investigated the effects of *in vivo* stimulation of innate immune cells with either murine G-CSF or GM-CSF on rituximab anti-tumor activity. NK cell-depleted SCID mice were inoculated by tail vein injection (tvi) with $1x10^6$ Raji cells on day 0. Animals were divided into three groups to receive four intraperitoneal (ip) doses (from days +1 to +4) of placebo, 5μg/day of murine (m)-GM-CSF or 5μg/day of m-G-CSF. Changes in CD11b/CD18 expression were performed by flow cytometric analysis of peripheral blood effector cells collected from each mouse. Subsequently, animals were subdivided to receive **sequential** placebo or rituximab (10mg/kg) on days +5, +8, +11 and + 14 via tail vein injection. A separate set of experiments was performed to address dose-schedule of cytokine administration. Lymphoma-bearing SCID mice were divided into six cohorts: Group A received placebo; Group B received m-G-CSF; Group C received m-GM-CSF; Group D received rituximab, Group E received concurrently m-GCSF and rituximab; and Group F received concurrent m-GM-CSF and rituximab. MAb doses (10mg/kg) were administered via tvi on days +3, +7, +11 and +15. Murine cytokines were given **concurrently** via ip injection two consecutive days before each dose of rituximab. The end point of the study was overall survival.

Treatment of lymphoma bearing SCID mice with rituximab resulted in significant higher anti-tumor activity and longer survival when compared with placebo controls. The median survival time for rituximab treated animals was 41 days (95%C.I. 30-52) in contrast to a median survival of 20 days (95% C.I. 20-20) for those animals receiving placebo (log rank test $P < 0.001$). No significant anti-tumor activity was observed in animals treated with either G-CSF or peg-GM-CSF alone, and the median survival was similar to control mice. The median survival for G-CSF treated SCID mice was 20 days (95% C.I. 19-20) and 21 days (95% C.I. 20-22) for those treated with peg-GM-CSF ($P= 0.20$).

The administration of murine G-CSF for two consecutive days prior to mAb therapy enhanced the anti-tumor activity of rituximab and doubled the mean survival of lymphoma-bearing mice. Statistically, significant differences were observed between animals treated with rituximab and G-CSF + rituximab. The mean survival time of animals treated with G-CSF and rituximab was longer [84 days (95% C.I. 75 to 93] than those treated with rituximab monotherapy [mean survival of 51 days (95%C.I. 37-64), log rank test P = 0.0036). After a median follow up time of three months, the median survival time for animals receiving rituximab in combination with G-CSF was not reached. (Figure 2A and 2B)

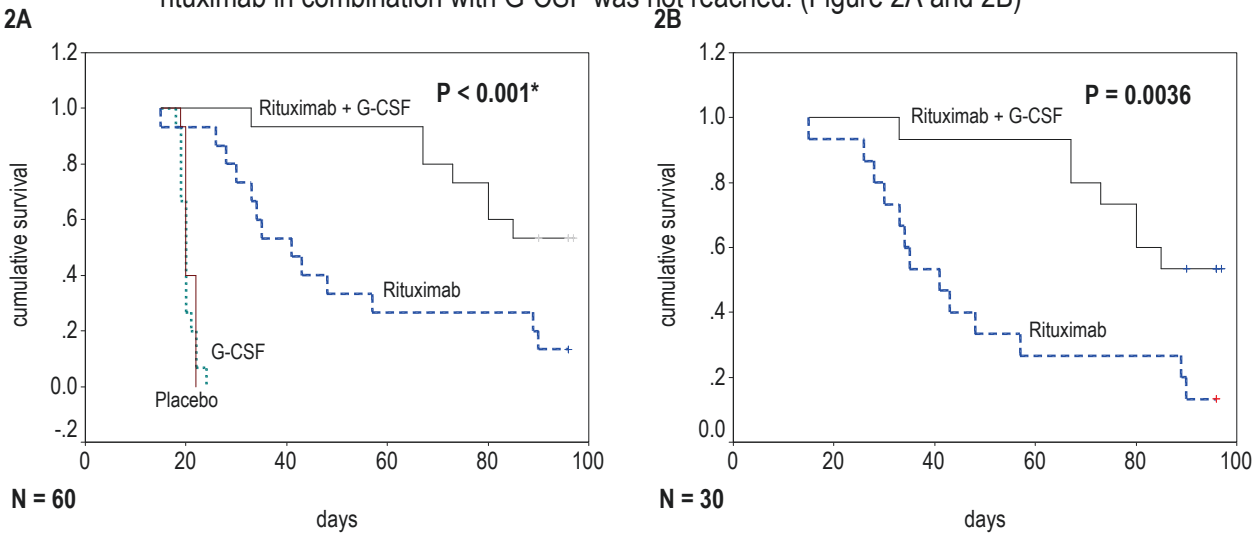

Figure 2. Kaplan Meier analysis of cumulative survival of SCID mice bearing raji lymphoma xenographs treated with rituximab and/or G-CSF. Groups of 15 NK cell depleted SCID mice were inoculated with 1 x 10^6 raji cells via tail vein injection (iv) [day 0] and treated either with placebo, rituximab (10mg/kg iv on days +5, + 9, +13 and +17), G-CSF (10μg/dose on days +3, +4, +7, +8, +11,+12, +15 and +16), or rituximab (10mg/kg iv on days +5, + 9, +13 and +17) in combination with G-CSF(10μg/daily for two consecutive days before each rituximab dose) and analyzed for survival as function of time. Significant anti-tumor activity was observed among animals treated with rituximab or rituximab in combination with G-CSF (3A). The administration of G-CSF for two consecutive days prior to each mAb dose resulted in a statistically significant enhancement in the biological activity of rituximab. The median survival of rituximab treated lymphoma bearing mice was only 41 days. On the other hand, after a follow up period of 3 months, the median survival time for animals treated with rituximab in combination with G-CSF has not been reach (log rank P = 0.0036) (3B)

The median survival for lymphoma-bearing SCID mice treated with rituximab and peg-GM-CSF was longer [62 days (95% C.I. 47-77)] when compared to rituximab treated animals [41 days (95%C.I. 30-52)]. Despite a trend towards improved survival, differences between rituximab-treated animals and those receiving the concurrent administration of rituximab in combination with peg-GM-CSF did not reach statistical significance (log rank P = 0.16). (Figure 3A and 3B)

After a follow up period of three months, survival rates were the highest for animals treated with rituximab and G-CSF (53.3%) when compared to animals treated with rituximab alone (13.3%) or in combination with peg-GM-CSF (26.67%). Pathological examination of all surviving animals at the end of the study failed to demonstrate any residual disease.

The administration of m-GCSF or m-peg-GM-CSF for two consecutive days led to a significant increase in the number of circulating white cell blood (WBC), specifically granulocytes of lymphoma bearing SCID mice. Animals receiving m-GCSF had a higher mean WBC count of 7.58 x 10^3/mm^3 (std. +/- 3.85 x 10^3/mm^3) than unstimulated SCID mice in which the mean WBC was 0.6533 x 10^3/mm^3 (std. +/- 0.23 x 10^3/mm^3]) (chi-square P < 0.001). In addition and to a lesser degree, animals that received peg-m-GM-CSF had a higher mean peripheral WBC count of 3.5 x 10^3/mm^3 (std. 1.3 x10^3/mm^3) (chi-square P < 0.001).

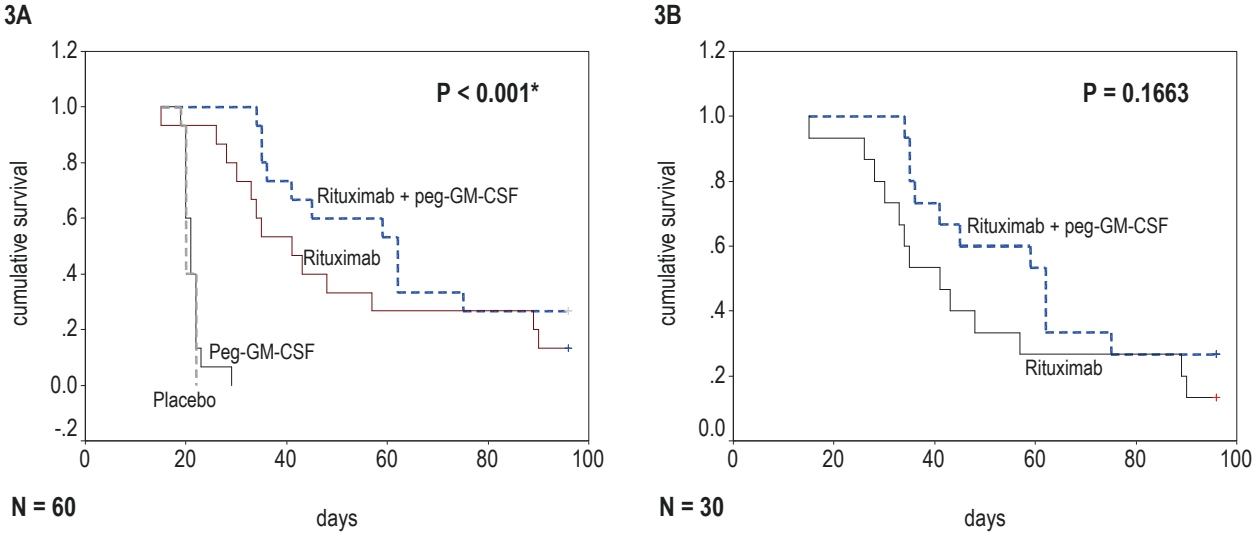

Figure 3. Kaplan Meier analysis of cumulative survival of SCID mice bearing raji lymphoma xenographs treated with rituximab or peg-GM-CSF. Groups of 15 NK cell depleted SCID mice were inoculated with 1 x 10^6 raji cells via tail vein injection (iv) [day 0] and treated either with placebo, rituximab (10mg/kg iv on days +5, + 9, +13 and +17), peg-GM-CSF (10μg/dose on days +3, +4, +7, +8, +11,+12, +15 and +16), or rituximab (10mg/kg iv on days +5, + 9, +13 and +17) in combination with peg-GM-CSF(10μg/daily for two consecutive days before each rituximab dose) and analyzed for survival as function of time. Treatment with rituximab was effective in arresting lymphoma growth and prolonging the survival of raji lymphoma xenographs when compared to placebo or peg-GM-CSF (4A). The addition of peg-GM-CSF to rituximab therapy resulted in longer median survival (62 days) when compared to rituximab single agent (median survival time of 41 days). However the difference did not reach statistical significance (log rank P = 0.1663) (4B)

Differences were primarily due to the number circulating granulocytes. The mean absolute neutrophil count (ANC) was higher among SCID mice treated with either m-G-CSF (4.98 +/- 2.7 x10^3/mm^3) (chi-square P < 0.001) or m-peg-GM-CSF (1.97 +/- 1.41 x10^3/mm^3) than in animals were unstimulated (0.38 +/- 0.26 x10^3/mm^3) (chi-square P = NS).

Flow cytometric analysis performed from whole blood collected 48 hours after the administration of m-GSF or peg-m-GM-CSF show phenotypic changes in the expression of activation markers on granulocytes. SCID mice treated with either cytokine (G-CSF or peg-GM-CSF) had a higher expression of CD11b/CD18 when compared to placebo controls. The up-regulation of CD11b/CD18 was restricted to Gr-1 positive cells (neutrophils). The mean percentage of circulating neutrophils

expressing CD11b/CD18 isolated from control mice was lower (46.81% +/- 6.73) than G-CSF primed (96.63% +/- 1.7) or peg-GM-CSF (88.21 +/- 5.1) [Figure 4]. On the other hand, no significant changes were observed in the expression of L-selectin (CD62)

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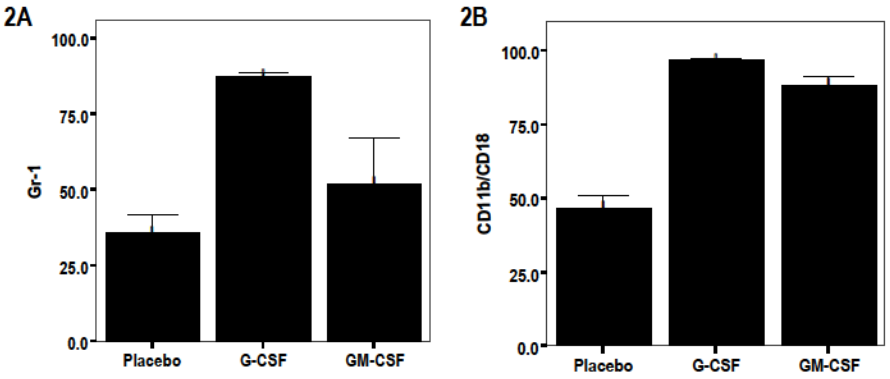

Figure 4. Flow cytometric analysis of peripheral blood collected from SCID mice show differences in the expression of activation markers among neutrophils treated with murine cytokines. A significantly higher number of granulocytes (Gr-1 + cells) were observed among animals receiving murine G-CSF and to a lesser degree murine peg-GM-CSF. In addition, the surface expression of the activation marker CD11b/CD18 was upregulated in lymphoma bearing mice treated with cytokines when compared to control animals.

In summary, the results of these mouse experiments demonstrate the promise of stimulating the immune effector cells (neutrophils) using G-CSF in order to enhance the biological anti-tumor activity of rituximab. The molecular basis for the effects of G-CSF on the anti-tumor activity of rituximab is yet to be fully defined. However, an increase in the trafficking of neutrophils into the tumor bed and enhancement of rituximab-mediated ADCC via upregulation of CD11b/CD18 are believed to play a significant role. Other contributing factors such as the production of free oxygen radical species, complement activation by alternative pathways, cytokine "storms" following cytokine/rituximab therapy, and the upregulation of CD20 antigen by pharmacologically achievable doses of G-CSF needs to also be considered. Our data support the basis for the development of clinical trials exploring the combination of rituximab in combination with G-CSF/PEG-Filgrastim.

1.5 Clinical Experience with Rituximab in Combination with G-CSF

Clinically, the combination of G-CSF with rituximab has been studied in a recently published phase I/II clinical trial. Patients with indolent and intermediate-grade NHL received G-CSF at 5μg/kg/dose for three consecutive days starting 48 hours prior to each rituximab infusion. Rituximab was administered intravenously at

375mg/m^2 weekly for four doses. The combination of G-CSF and rituximab was well tolerated and while the response rate was similar to historical rituximab monotherapy treated controls, the duration of response was longer [25]. Similarly to what we observed, G-CSF therapy resulted in an incremental increase in the neutrophil count of patients prior to rituximab therapy [25]. Notably, in contrast with our animal study results, a downregulation of CD11b/CD18 expression in circulating neutrophils were observed at 24 hours prior to rituximab therapy. Differences in the expression of CD11b/CD18 observed could be the result of inter-species variations in the degree and speed of neutrophil responsiveness and migration.

The molecular basis for the effects of G-CSF on the anti-tumor activity of rituximab need to be fully defined. However, an increase in the trafficking of neutrophils into the tumor bed and enhancement of rituximab-mediated ADCC via upregulation of CD11b/CD18 likely play significant roles. Recently studies conducted in CD11b/CD18 deficient mice demonstrated that the capacity of neutrophils to induce ADCC against antibody-coated melanoma cells was impaired [26]. Another group of investigators have demonstrated changes in CD20 antigen expression following *in vitro* exposure to various cytokines [27]. In our studies we could not demonstrate a significant upregulation of surface CD20 following *in vitro* exposure of lymphoma cells to either G-CSF or GM-CSF. Discrepancies between our results and those reported by Dr. Venugopal could be related to differences in cytokine dose, timing, and use of cell lines versus freshly isolated patient specimens. A better understanding in the physiological events that occur following the administration of G-CSF and rituximab will hopefully lead to an improvement in rituximab-associated anti-tumor activity in patients with B-cell lymphoma.

1.6 Clinical Experience with Pegfilgrastim

Neulasta® (pegfilgrastim) is a covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol. Both Filgrastim and pegfilgrastim are Colony Stimulating Factors that act on hematopoietic cells by binding to specific cell surface receptors thereby stimulating proliferation, differentiation, commitment, and end cell functional activation. Studies on cellular proliferation, receptor binding, and neutrophil function demonstrate that Filgrastim and pegfilgrastim have the same mechanism of action. Pegfilgrastim has reduced renal clearance and prolonged persistence in vivo as compared to Filgrastim.

Neulasta® is indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia.

Clinical Experience
Pegfilgrastim was evaluated in three randomized, double-blind, controlled studies. Studies 1 and 2 were active-controlled studies that employed doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2 administered every 21 days for up to 4 cycles for the treatment of metastatic breast cancer.[27,28] Study 1 investigated the utility of a fixed dose of pegfilgrastim. Study 2 employed a weight-adjusted dose. In the absence of growth factor support, similar chemotherapy regimens have been reported to result in a 100% incidence of severe neutropenia (absolute neutrophil count [ANC] < 0.5 x 10^9/L) with a mean duration of 5-7 days, and a 30%-40% incidence of febrile neutropenia. [29]

In study 1, 157 subjects were randomized to receive a single subcutaneous (SC) dose of 6 mg of pegfilgrastim on day 2 of each chemotherapy cycle or Filgrastim at 5 mcg/kg/day SC beginning on day 2 of each cycle. In study 2, 310 subjects were randomized to receive a single SC injection of pegfilgrastim at 100 mcg/kg on day 2 or Filgrastim at 5 mcg/kg/day SC beginning on day 2 of each cycle of chemotherapy.

Both studies met the primary objective of demonstrating that the mean days of severe neutropenia (ANC<0.5 x 10^9/L) of pegfilgrastim-treated patients did not exceed that of Filgrastim-treated patients by more than one day in cycle 1 of chemotherapy. The rates of febrile neutropenia were 13% and 9% for pegfilgrastim vs. 20% and 18% for Filgrastim in studies 1 and 2, respectively. [27,28] Other secondary endpoints included days of severe neutropenia in cycles 2-4, the depth of ANC nadir in cycles 1-4, and the time to ANC recovery after nadir. In both studies, the results for the secondary endpoints were similar between the two treatment groups.

Study 3 was a randomized, double-blind, placebo-controlled study that employed docetaxel 100 mg/m^2 administered every 21 days for up to 4 cycles for the treatment of metastatic or non-metastatic breast cancer. In this study, 928 patients were randomized to receive a single subcutaneous injection of Neulasta® 6 mg or placebo on day 2 of each chemotherapy cycle. Study 3 met the primary objective of demonstrating that the incidence of febrile neutropenia (defined as temperature $\geq$ 38.2°C and ANC $\leq$ 0.5 x10^9/L) was lower for Neulasta®-treated patients as compared to placebo-treated patients (1% versus 17%, p < 0.001). The incidence of hospitalizations (1% versus 14%) and IV anti-infective use (2% versus 10%) for the treatment of febrile neutropenia were also lower in the Neulasta®-treated patients compared with the placebo-treated patients. [30]

The safety and efficacy of once-per-cycle pegfilgrastim was also found to be comparable to daily Filgrastim in phase 2 studies in patients with non-small cell lung cancer being treated with carboplatin and paclitaxel and patients with non-Hodgkin's lymphoma (NHL) or Hodgkin's lymphoma being treated with ESHAP (etoposide, methylprednisolone, high-dose cytarabine, cisplatin) or CHOP

(cyclophosphamide, doxorubicin, vincristine, prednisone) chemotherapy. [31, 32, 33]
Refer to the Package Insert for additional information on the clinical pharmacology, pharmacokinetics and precautions of pegfilgrastim.

2.0 OBJECTIVES

2.1 Primary objective:

To evaluate the safety of Pegfilgrastim in combination with rituximab in patients with untreated or relapsed/refractory follicular, small lymphocytic lymphoma (SLL) or marginal zone lymphoma (MZL).

2.2 Secondary objectives:

To evaluate the efficacy (including overall response rate and durability of objective responses) of Pegfilgrastim in combination with rituximab in patients with untreated or relapsed/refractory follicular, small lymphocytic lymphoma (SLL) or marginal zone lymphoma (MZL).

To evaluate functional and phenotypic characteristics of host neutrophils undergoing treatment with Pegfilgrastim and rituximab

To evaluate changes in CD20 antigen expression and density of expression in patients receiving Pegfilgrastim and rituximab

To evaluate changes in serum levels of tumor necrosis factor (TNF), interferon alpha (INFα) and free radical levels in patients undergoing treatment with Pegfilgrastim and rituximab

3.0. STUDY DESIGN OVERVIEW

This open-label, single-arm, single-center Phase II study will evaluate the safety, tolerability and efficacy of rituximab antibody in combination with Pegfilgrastim in patients with untreated or relapsing/refractory follicular, SLL or MZL. We expect to enroll 40 patients from Roswell Park Cancer Institute over thirteen years.

4.0. STUDY POPULATION

Eligible REAL disease classifications include untreated, or relapsed / refractory:

1. Follicular lymphoma; grade 1, 2, 3a
2. Small lymphocytic lymphoma.
3. Marginal zone lymphoma.

4.1 Patient Eligibility

4.1.1 Inclusion Criteria

Patients must meet the following criteria to be eligible for study admission:

- Untreated or relapsed/refractory follicular, small lymphocytic lymphoma or marginal zone lymphoma (i.e. no limit to number of prior treatments as long as patients meet other study criteria)
- ECOG performance status 0 or 1
- Age >18 years
- Measurable tumor size (at least one node measuring 4 cm^2 in bidimensional measurement)
- Expected survival of > 6 months.
- Prior rituximab or other monoclonal immunotherapy permitted and eligible for rituximab monotherapy.
- Full recovery from any significant toxicity associated with prior surgery, radiation therapy, chemotherapy, or immunotherapy.
- Absolute neutrophil count > 1.0 x 10^9/L
- Platelets > 50 x 10^9/L
- Patients may receive erythropoietin growth factors to maintain adequate hemoglobin levels ($\geq$ 8.0 mg/dl).
- Creatinine <1.5 x UNL
- Total bilirubin < 1.5 mg/dL (> 25.65 μmol/L).
- Aspartate aminotransferase < 5 x UNL
- Alkaline phosphatase < 5 x UNL
- Informed consent approved in institutional IRB
- CD20+ B-cell lymphoma

4.1.2 Exclusion Criteria

Patients will be excluded from the study based on the following criteria:

- Prior history of HIV-positivity (Routine HIV testing is required pre-treatment)
- Serious non-malignant disease (e.g. active uncontrolled bacterial, viral, or fungal infections) or other conditions which, in the opinion of the principal investigator would compromise other protocol objectives
- Presence of CNS lymphoma
- Chemotherapy within 4 weeks of the first scheduled study treatment
- Another primary malignancy (other than squamous or basal cell

carcinoma of the skin or in-situ carcinoma of the cervix) for which the patient has not been disease-free for at least five years.
- Major surgery, other than diagnostic surgery, within four weeks.
- Patients with NHL other than relapsed/refractory folicullar, MZL or SLL
- Patients must not have a history of cardiac disease, defined as New York Heart Association Class II or greater or clinical evidence of congestive heart failure.
- Concurrent use of other investigational agents
- Pregnant or breast feeding
- Subjects of reproductive potential who are not using adequate contraceptive precautions, in the judgment of the investigator
- Known hypersensitivity to any recombinant *E coli*-derived product, murine proteins, or any components of the study medications
- Concerns for the subject's compliance with the protocol
- Any premalignant myeloid condition or any malignancy with myeloid characteristics (e.g. myelodysplastic syndromes, acute or chronic myelogenous leukemia)
- Patient is currently enrolled in, or has not yet completed at least 30 days since ending another investigational device or drug trial

5.0 DOSE-SCHEDULE AND FORMULATION:

Enrolled patients will receive Pegfilgrastim 6mg subcutaneously (sq) 3 days before each dose of rituximab. Monoclonal antibody therapy will consist of rituximab (Rituxan®) administered intravenously (iv) at a dose of 375mg/m^2 every other week for four doses and after 8 weeks every 2 months for four additional doses. All patients are expected to receive a total of 8 doses of Pegfilgrastim and eight doses of rituximab (Rituxan®). The treatment will be administered over a total period of 39 weeks.

The standard administration of rituximab single agent is 375mg/m^2 weekly x 4. However various schedules had been studied in clinical trials. Two "extended" regimens were studied by The Minnie Pearl Cancer Center and by the Swiss Group for Clinical Cancer Research. The first group studied the administration of rituximab at the standard dose of 375mg/m^2 weekly x 4 follow by maintenance rituximab at the same dose weekly x 4 every 6 months for 2 years. The second group compared the standard regimens of rituximab versus an extended 8 regimen consisting of rituximab administered at 375mg/m^2 weekly x 4 follow by 4 additional doses of rituximab (375mg/m^2) every 8 weeks starting 12 weeks after the completion of the induction rituximab therapy. In both studies there was a prolongation in the time to treatment failure compared to rituximab historical controls. However a survival advantage was not observed.

In our current study we are seeking to study if by enhancing the innate immune system with pegfilgrastim, we can modulate rituximab activity without adding significant toxicity. Because of the pharmacokinetics of pegfilgrastim we decided to administer the first 4 doses of rituximab every 2 weeks instead of the standard weekly schedule. Based on pharmacokinetic data in early rituximab

clinical trials, the modification in the first four doses of rituximab should not affect rituximab levels as the half life of this antibody is extremely long. The additional four doses are administered as previously done by the Swiss Group. It is important from the safety stand point to note that the Swiss schedule has been adopted by several Oncologist in the United States as their preferred schedule given the safety profile and better anti-tumor activity over the standard schedule of four doses of rituximab.

Table 1. Dose-schedule administration of Pegfilgrastim in combination with rituximab

Week	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39
Rituximab	◙	◙	◙	◙				◙				◙				◙				◙
Pegfilgrastim	◙	◙	◙	◙				◙				◙				◙				◙
Pegfilgrastim will be administered sq 3 days (day 1 of each treatment week) before each dose of rituximab (day 4 of each treatment week)																				

Pegfilgrastim is supplied as a preservative-free solution containing 6 mg (0.6 mL) of Pegfilgrastim (10 mg/mL) in a single-dose syringe with a 27 gauge, 1/2 inch needle with an UltraSafe® Needle Guard.

Rituximab (Rituxan®) is a sterile, clear, colorless, preservative free liquid concentrate for intravenous (IV) administration. Rituximab is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single use vials. The product is formulated for IV administration in 9.0 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Sterile Water for Injection. The pH is adjusted to 6.5.

Premedication with acetaminophen 650 mg PO and diphenhydramine hydrochloride 25 mg IV. Premedication will be done before each rituximab dose. The initial dose rate at the time of the first rituximab infusion will be 50 mg/hr for the first hour. If no toxicity is seen, the dose rate will be escalated in 50 mg/hr increments in 30-minute intervals to maximum of 400 mg/hr. If the first dose of rituximab is well tolerated, the starting flow rate for administration of subsequent doses will be 100 mg/hr then escalated in 100 mg/hr increments at 30-minute intervals not to exceed 400 mg/hr.

6.0 COMPLIANCE

This study will be conducted in accordance with current U.S. Food and Drug Administration (FDA) Good Clinical Practices (GCPs), the Declaration of Helsinki, and local ethical and legal requirements.

7.0 PROCEDURES

7.1 Study Procedures by Visit (For a summary of the treatment procedures and evaluations see Appendix section)

7.1.1 Screening and Pretreatment Assessments

All patients must have signed the Informed Consent Form prior to initiation of any study-related evaluations. All screening procedures must be completed within 4 weeks of study enrollment and

initiation of treatment with Pegfilgrastim/rituximab. The following evaluations and procedures will be performed during the screening period:

- Medical history
- Physical exam (including lymph node, liver and spleen evaluation) and vital signs
- Height and body weight (BSA calculation)
- ECOG performance status
- CBC with 5-part differential and platelet count.
- Chemistry panel (electrolytes, albumin, BUN, creatinine, bilirubin, alkaline phosphatase, LDH, ALT, AST, calcium, phosphorous); in addition, peripheral blood sample will be sent for Bcl-2 detection
- Pregnancy test (if applicable)
- Bone marrow aspirate, biopsy and sample for markers, cytogenetic analysis, PCR for Ig gene rearrangement
- Lymph node biopsy and sample for surface antigen markers (e.g. CD20, CD19, etc.); PCR for Ig gene rearrangement in FL patients; and Bcl-2 detection for patients with accessible and enlarged lymph node. As long as they do not have any medical contraindications for biopsy as determined by the treating physician. For patients with no accessible tumor lesions or with medical contraindications for surgical procedure, archived pathological material will be analyzed for confirmation of diagnosis, CD20 expression and in cases of FL for IgH gene rearrangement and Bcl-2 studies Beta 2 microglobulin (β2M)
- Urinalysis
- Tumor measurements (bidimensional by physical examination and/or radiological studies
- Imaging studies for disease assessment (a minimum of one and a maximum of six sentinel lesions will be selected for determining the efficacy of the study treatment) within 4 weeks prior to treatment.
    - PET scan, CT scan or MRI of chest, abdomen, and pelvis (and when appropriate, neck).
- Quantitative immunoglobulins (IgG, IgA, IgM)
- Screening of patients for HBV and HCV

7.1.2. <u>Assessments during Treatment Period (Weeks 1,3,5,7, 11, 15, 23, 27, 31, 39 and 43)</u>

Patients with easily accessible lymphomatous lesions will be requested to undergo an excisional biopsy after completion of the first dose of Pegfilgrastim and rituximab (within 24 hrs after completion of the antibody infusion). Clinical and laboratory evaluations will be performed before each rituximab treatment for weeks 1,3,5,7, 15, 23, 31 and 39. Imaging evaluations will be performed on week 11, before entering into the extended rituximab schedule, at the middle of the extended rituximab schedule (week 27) and at week 43 following completion of therapy.

The following evaluations and procedures will be performed during the treatment period at each time interval as described above:

- Physical exam (including lymph node, liver and spleen evaluation) and vital signs
- Body weight
- ECOG performance status
- CBC with 5-part differential and platelet count.
- Chemistry panel (electrolytes, albumin, BUN, creatinine, bilirubin, alkaline phosphatase, LDH, ALT, AST, calcium, phosphorous)
- Imaging studies for disease assessment (week11) prior to the fifth dose of pegfilgrastim and rituximab, in the middle of the extended schedule (week 27) and approximately 1 month after the last dose of study treatment [week 43]
  - PET scan, CT scan (or when necessary, MRI) of chest, abdomen, pelvis, (and neck if appropriate)
  - The sentinel lesions chosen at baseline will be assessed at the times indicated above. If the patient has a response (PR or CR), the patient must be reassessed 1 to 4 months later to confirm the response. Bone marrow studies will be repeated at one month after completion of therapy [i.e., week 43] in all patients with lymphomatous involvement evaluate "complete response" status in patients achieving "natal" CR on exam/radiological studies
- Record adverse events and concomitant medications (throughout the treatment period)

7.1.3. End of the study assessments (Week 43 studies).

The following tests and/or data collection must be performed or obtained during week 43 and four weeks or greater after completing all therapy.

- Physical exam (including lymph node, liver and spleen evaluation) and vital signs
- ECOG performance status
- CBC with 5-part differential and platelet count.
- Chemistry panel (electrolytes, albumin, BUN, creatinine, bilirubin, alkaline phosphatase, LDH, ALT, AST, calcium, phosphorous)
- Bone marrow aspirate, biopsy and sample for previously measured antigen markers, for Ig gene rearrangement (and Bcl-2 by PCR in FL patients only)
- Beta 2 microglobulin (β2M) (if elevated pre-therapy)
- Tumor measurements (using same methodology utilized pre-therapy)
- Quantitative immunoglobulins (IgG, IgA, IgM)

7.1.4 Assessments during Follow-up Period

All patients will be followed for assessment of time to progression and survival only, for 4 years (48 months) after the last study treatment.

- The following assessments and/or studies must be performed or obtained

every 4 months during the first year of follow-up; then every 6 months during the second and third years of follow-up; and yearly thereafter. History and Physical Examination

- ECOG performance status
- Imaging studies for disease assessment. Response classifications (definitions) are fully described in Appendix.
  - PET scan if clinically indicated, CT scan (or MRI) of chest, abdomen and pelvis (and when appropriate, neck). CT will be performed during the first two years of follow-up only.
- Laboratory studies
  - Hematology: CBC with differential and platelet count. In the event of Grade 3 or 4 hematologic toxicity, follow-up evaluations will be performed as clinically indicated until the toxicity has resolved.
  - Serum Chemistries: creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, glucose, AST (SGOT), calcium, phosphate, sodium, potassium, and BUN.

The following studies will be performed annually during the 4 year (48 month) follow-up period:

- Laboratory studies
  - Peripheral blood (and bone marrow samples when available) for bcl-2 testing if the baseline assessment was positive for this marker

7.2 Patient Discontinuation

Patients may choose to withdraw from the study at any time.

The Principal Investigator for the following reasons may discontinue patients from the study:

- Adverse experiences
  - Patients with persistent Grade ≥3 non-hematologic toxicity or any significant adverse event that, in the opinion of the investigator, compromises the patient's ability to participate in the study will be withdrawn from the study.
  - Patients requiring treatment interruption for more than 3 weeks will be removed from the study.
- Disease progression
  - Patients with progressive disease (as defined in Appendix C) or clinically significant deterioration at any time during the study will be withdrawn from the study.
- Investigator judgment
  - Patients may be withdrawn from the study if, in the opinion of the investigator, it is not in the patient's best interest to continue, e.g., an adverse experience, intercurrent illness, pregnancy, lack of efficacy, etc.

All patients discontinuing the study will be requested to complete the Week 11 and 43 assessments.

7.3 Study Discontinuation

Reasons for terminating the study may include the following:

- The incidence or severity of adverse events in this or other studies indicates a potential health hazard to patients
- Patient enrollment is unsatisfactory
- Data recording is inaccurate or incomplete

7.4 Safety Evaluations

The primary objective of this study will be to evaluate the safety of pegfilgrastim in combination with rituximab in patients with relapsed / refractory follicular, small lymphocytic or marginal zone lymphoma.

Safety will be assessed by

- continuous monitoring for adverse events
- laboratory studies prior to each cycle of therapy
- vital signs
- physical examinations
- restaging prior as indicated above

All adverse events will be continuously monitored until resolution.

Adverse events that may be seen in this study include:

- Events related to the administration of rituximab, such as asthenia, infusion-related symptoms (e.g. fever, chills, and hypotension) most common with the first infusion, and rarely mild thrombocytopenia and neutropenia.
- Events related to the administration of pegfilgrastim, such as bone pain.

7.5 Efficacy Evaluations

As a secondary objective this study will evaluate the antitumor efficacy of rituximab in combination with pegfilgrastim in this patient population.

Efficacy assessments will include

- clinical evaluation of disease (to be performed during week number 11,27 and 43
- radiologic evaluation of the extent of disease following therapy (to be performed on weeks 11, 27 and 43) will be used to assess response according to the standard World Health Organization (WHO) response criteria (Appendix).
    - PET scans, computerized tomography (CT) scans, or magnetic resonance imaging (MRI). CT scans will be performed during the first two years of follow-up only.
    - other relevant x-ray studies, will be performed
- periodic (during week 43 and annually thereafter for four years) assessment of bcl-2 (chromosome 14;18 translocation) by PCR in peripheral blood (and when possible, in bone marrow) for those follicular lymphoma patients testing positive for this genetic marker pretreatment

Efficacy parameters to be evaluated include:

- overall response rates
- complete response (CR) rates
- partial response (PR) rates
- time to progression

7.6. Correlative studies description:

In this particular study we will study the mechanisms by which pegfilgrastim may improve rituximab activity. In our preclinical studies using lymphoma cells lines and lymphoma xenografts, we found that G-CSF increases not only the number of neutrophils but also the expression of adhesion molecules such as CD11b that were partially responsible for the improvement in rituximab activity. Therefore we will conduct similar studies in enrolled patients that may validate our pre-clinical data.

Various laboratory parameters will be analyzed for each patient enrolled in the study before treatment (baseline) and before each dose of rituximab. Laboratory studies will study the changes in neutrophils induced by Pegfilgrastim and will be correlated with clinical response and duration of response in each patient completing the study.

1. Phenotype changes in neutrophils: Flow cytometry will be analyzed for changes in the surface expression of CD11b/CD18, CD16, CD32 and CD64. Differences in antigen density will be determined by mean fluorescence units.
2. Changes in the oxidative burst by neutrophils stimulated with Pegfilgrastim as measured by flow cytometry studies and measurement of free radicals in serum from patients treated with rituximab and Pegfilgrastim. Oxidative burst will be measured as by flow cytometry analysis.
3. Functional Assays in vitro: ADCC assays: The capacity to lyse tumor cells in the presence of rituximab by Peripheral Blood mononuclear cells (PBMC's) of each patient will be studied by standardized ^{51}Cr release assays. Briefly 2 $x10^6$ lymphoma cells (Raji cells) will be labeled with ^{51}Cr. Labeled cells will be then exposed to rituximab or isotype control (10μg/ml) and peripheral blood mononuclear cells isolated from each patient be at the intervals described above (Effector: Target ratio 40:1)^{51}Cr-release was measured and the percentage of lysis was calculated using the following formula:

   % Lysis = [CPM sample – CPM background/ CPM positive control – CPM background] * 100.
4. Monitor serum levels of INFα and TNF in patients treated with rituximab in combination with Pegfilgrastim. Cytokines levels will be measured by flow cytometry in the Roswell Park Cancer Institute Flow Cytometry Department.

In addition, patients with easily accessible lymphomatous lesions will be requested to undergo an excisional biopsy after completion of the first dose of Pegfilgrastim and rituximab (within 24 hrs after completion of the antibody infusion). Tissue will be analyzed for the following:

1. Infiltration of neutrophils into the tumor bed
2. Changes in CD20 expression as determined by immunohistochemistry (L26 staining), flow cytometry, and Western blotting studies for evaluation of cell viability and evidence of apoptosis; comparison to pre-treatment lymphoma biopsy specimens
3. Pathological examination

8.0 ADVERSE EVENT REPORTING

In the event of an adverse experience, the first concern will be for the safety of the patient. Timely, accurate, and complete reporting and analysis of safety information from clinical trials are crucial

for the protection of patients, investigators, and the Sponsor, and is mandated by regulatory agencies worldwide. Adverse events will be classified according to the NCI Common Toxicity Criteria using the Toxicity Scale version CTCAE v3.0.

8.1. Definitions

8.1.1 Adverse Event Classification Definitions

Adverse Event:

Any untoward medical occurrence in a patient or clinical investigation patient administered a pharmaceutical product and which does not necessarily have a causal relationship with this treatment. An AE can therefore be any unfavorable and unintended sign (including an abnormal finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not related to the medicinal (investigational) product.

This includes any occurrence that is new in onset or aggravated in severity or frequency from the baseline condition, abnormal results of diagnostic procedures including laboratory test abnormalities which are considered AEs if they:

- result in discontinuation from the study,
- require treatment or any other therapeutic intervention,
- require further diagnostic evaluation (excluding a repetition of the same procedure to confirm the abnormality),
- are associated with clinical signs or symptoms judged by the investigator to have a significant clinical impact.

Serious Adverse Event (SAE):

Any untoward medical occurrence that at any dose:

- results in death,
- is life-threatening (The patient was at risk of death at the time of the event. It does not refer to an event which hypothetically might have caused death if it were more severe),
- requires inpatient hospitalization or prolongation of existing hospitalization,
- results in persistent or significant disability/incapacity,

or

- in a congenital anomaly/birth defect.

Note: Medical and scientific judgment should be exercised in deciding whether expedited reporting is appropriate in other situations, such as important medical events that may not be immediately life-threatening or result in death or

hospitalization but may jeopardize the patient or may require intervention to prevent one of the outcomes listed in the definition above.

Unlisted (Unexpected) Adverse Event:

An AE, the nature or severity of which is not consistent with the applicable product information (e.g. Investigator's Brochure) for an unapproved investigational product or package insert/summary of product characteristics for an approved product.

Associated With the Use of the Drug:

An AE is considered associated with the use of the drug if the attribution is possible, probable or very likely.

8.1.2. Attribution Definitions

Not related

An AE which is not related to the use of the drug.

Unlikely

An AE for which an alternative explanation is more likely - e.g. concomitant drug(s), concomitant disease(s), and/or the relationship in time suggests that a causal relationship is unlikely.

Possible

An AE which might be due to the use of the drug. An alternative explanation - e.g. concomitant drug(s), concomitant disease(s) - is inconclusive. The relationship in time is reasonable; therefore the causal relationship cannot be excluded.

Probable

An AE which might be due to the use of the drug. The relationship in time is suggestive (e.g. confirmed by dechallenge). An alternative explanation is less likely - e.g. concomitant drug(s), concomitant disease(s).

Likely

An AE which is listed as a possible adverse reaction and cannot be reasonably explained by an alternative explanation - e.g. concomitant drug(s), concomitant disease(s). The relationship in time is very suggestive (e.g. it is confirmed by dechallenge and rechallenge).

8.2 Procedures

8.2.1 All Adverse Events

All AEs and SAEs experienced by enrolled patients for whom study drug is provided must be reported according to this procedure. All AEs will be reported between the first trial related procedure and the last dose of study drug. Any AE that is ongoing must be followed for 90 days following the last trial related procedure. Those meeting the definition of SAE must be reported between the

first trial related procedure and 90 days after the last trial related procedure using the SAE Form. Patients should voluntarily report any AEs or in response to general, non-directed questioning (e.g., "How has your health been since the last visit?"). For each AE volunteered by the patient, the investigator should obtain all the information required to complete the AE page of the CRF, in accordance with the guidelines that accompany it.

All AEs, regardless of seriousness, severity, or presumed relationship to study therapy, must be recorded using medical terminology in the source document and on the CRF. Whenever possible, diagnoses should be given when signs and symptoms are due to a common etiology (e.g., cough, runny nose, sneezing, sore throat, and head congestion should be reported as "upper respiratory infection"). Investigators must record on the CRF their opinion concerning the relationship of the AE to study therapy. All measures required for AE management must be recorded in the source document and reported according to Sponsor instructions.

The principal investigator assumes responsibility for appropriate reporting of AEs to the regulatory authorities. The Sponsor will also report to the investigator all serious AEs that are unlisted and associated with the use of the drug. The investigator must report these events to the appropriate Institutional Review Board (IRB) in accordance with local regulations.

8.2.2 Serious Adverse Events

All SAEs occurring during clinical trials must be reported within one working day (24 hours) to IRB. This includes but is not limited to type, grade, action taken and relationship to the study medications.

The cause of death of a patient in a clinical trial, whether the event is expected or associated with the study drug, is an SAE.

The initial report of an SAE may be made by telephone or facsimile (FAX). The investigator must provide the minimal information: i.e. protocol number, patient's initials and date of birth, patient number or medication code number, period of intake, nature of the adverse event and investigator's attribution.

All oral reports of an SAE must be confirmed within three days by a written, more detailed report and signed by the investigator. For this purpose, the Sponsor will provide the investigator with the Serious Adverse Event Form.

All SAEs that have not resolved by the end of the study, or that have not resolved upon discontinuation of the patient's participation in the study, must be followed until either:

- the event resolves, or
- the event stabilizes, or
- the event returns to baseline, if a baseline value is available, or

- the event can be attributed to other than the study drug, or to other than study conduct.

8.2.3 Pregnancies

Pregnancies occurring between the first trial-related procedure and 90 days after the last trial-related procedure must be reported to IRB within one working day after the investigator or study staff has gained knowledge of them, using the Serious Adverse Event Form. Follow-up information regarding the outcome of the pregnancy and any postnatal sequelae in the infant will be requested from Amgen's Drug Safety and Surveillance (DSS).

All patients who become pregnant during participation in this trial must be promptly withdrawn from the trial.

Although pregnancy is not an adverse event, manufacturers should be notified of pregnancies occurring on study.

8.3 Reporting Procedures for Serious Adverse Events

All serious adverse events related to study drug must be reported to Amgen Global Safety within one working day of discovery or notification of the event. Use a MedWatch form or appropriate institutional form for reporting adverse events to Amgen. Initial serious adverse event information and all follow-up information must be recorded on a Serious Adverse Event form and faxed using the Amgen Serious Adverse Event Report Cover Page to:

Amgen Global Safety (Fax: 888-814-8653 / Phone: 866-264-3650)

The investigator should notify the IRB of serious adverse events occurring at the site, in accordance with local procedures. All serious and medically significant adverse events considered related to pegfilgrastim by the investigator will be followed until resolved or considered stable.

In addition, any serious adverse events related to the use of Rituximab will be also need to be reported to Genentech/Biogen Idec: Monic Stuart, MD, Genentech, 1 DNA Way, South San Francisco, California 94080 (Fax 650-225-5862 / Phone: 650-467-1256)

8.4 Internal Data Safety and Monitoring

The same monitoring process in effect for all RPCI investigator initiated studies will monitor this trial. All patients on study are reviewed for any toxicity or other untoward events during the Data Safety and Monitoring Board (DSMB). The primary responsibilities of the DSMB are to 1) periodically review and evaluate the accumulated study data for participant safety, study conduct and progress, and, when appropriate, efficacy, and 2) make recommendations to the IRB concerning the continuation, modification, or termination of the trial.

The first review is planned to be conducted after the first six patients complete the proposed treatment. Patient recruitment, accrual and retention will also be discussed at DSMB meetings as needed. Toxicity is reported to the DSMB.The

principal investigator will continuously monitor the study and will submit safety and monitoring reports to the institution IRB and DSMB as required.

If any literature becomes available which suggests that conducting this trial is no longer ethical, the study will be terminated and the IRB will be notified of the new findings. The IRB will be notified of any change in the risk/benefit ratio that would affect whether the study should continue. All SAEs will be reported to the IRB according to the established guidelines. A cumulative summary of all AEs occurring on this study will also be submitted to the IRB with the annual periodic review. Toxicity is reported, as required, to the FDA and study sponsors. All study data reviewed and discussed during these meetings will be kept confidential. Any breech in patient confidentiality will be reported to the IRB.

9.0 STATISTICAL METHODS

9.1 Analysis of the Conduct of the Study

Patient enrollment, major protocol deviations and violations, withdrawals from the study, and adherence to study medication will be summarized by the study center.

9.2 Baseline Demographics

The demographic and baseline data will be summarized by participating center. Means and standard deviations will be used for the demographic continuous variables. Absolute and relative frequencies will describe categorical variables.

Demographic and baseline variables are the following:

- Sex
- Age
- Weight (kg)
- Height (cm)
- Body surface area (BSA = Square Root (weight x height)/3600) (m^2)
- Performance status
- Histologic classification
- Free radical species levels
- Levels of INFα and TNF
- CD11b/CD18, CD62, , CD56, FcγIII and FcγII receptor polymorphism status of PMNs
- % of cell lysis by ADCC pre and post pegfilgrastim and rituximab

9.3. Safety

The study will be conducted in two parts. Evaluation of the safety of rituximab and pegfilgrastim will be done after the collection of the first twenty patients (First part of the study). All patients who complete at least one dose of pegfilgrastim will be considered evaluable for safety analysis. Patients who are not evaluable for safety will be replaced. The first twenty patients evaluable for safety will be used determine if the combination of rituximab and pegfilgrastim is tolerable. This number of patients is sufficient to determine the safety of the combination. The combination of rituximab and pegfilgrastim will be deemed tolerable if at least 75% of patients (15/20) tolerate the combination as measured by the absence of dose limiting toxicity (DLT). DLT is defined as any grade $\geq$3

hematological (excluding isolated lymphopenia or leukopenia) or non-hematological toxicity at any time during protocol therapy that is related to the study drug combination. Isolated lymphopenia is expected following rituximab therapy as a result of peripheral B-cell depletion. The collection of twenty additional patients will take place only if this standard is met. All 40 subjects will be reviewed for safety.

Previously, other investigators had investigated the safety and efficacy of rituximab in combination with G-CSF in patients with relapsed/refractory NHL. In that study the combination of rituximab and G-CSF was well tolerated. Adverse events, which occurred in 25/26 patients, were mainly consisted of (grade I/II) fever (29%) and allergic reactions (19%) [25]. In the present clinical study if during the first phase of enrollment, four (20%) patients experience dose-limiting toxicity (DLT) accrual of patients will stop and the combination of rituximab and pegfilgrastim will be determined intolerable. Safety will be monitored as described in section 7.

9.4 Efficacy

Response to treatment for all patients will be assessed at weeks 11, 27, and 43. Responses will be confirmed within 4 months after completion of all therapy for all patients. The **primary efficacy** endpoint for this study is the overall response rate (ORR) at weeks 11, 27, and 43. Secondary endpoints are the complete response (CR), partial response (PR) rates at weeks 11, 27, and 43, the time to disease progression, and survival. The primary and secondary efficacy endpoints are largely based on radiological assessment of the tumor. In addition, efficacy endpoints will be compared to historical previously published clinical data with rituximab given as a single agent (i.e. historical controls).

9.4.1. Methodology

The primary patient population will be based on the intent-to-treat approach and will include all enrolled patients. Patients who die as the result of disease progression or related complications before a tumor evaluation, or who take concomitant chemotherapeutic medication not specified in the protocol, or who withdraw from the study will be considered treatment failures.

The secondary patient population will include patients who complete the full treatment cycle. The full treatment cycle includes eight doses of rituximab and eight doses of pegfilgrastim administered over a 39 week period as described in table 1.

Primary and Secondary analyses. After the 43-week data from all patients enrolled have been entered into the database, an analysis of the response rates will be conducted. The ORR, CR and PR rates will be estimated for all enrolled patients (intent-to-treat approach). Two-sided 95% exact confidence intervals around the estimates will be calculated.

The estimate and the 95% exact confidence intervals of the ORR rate and the CR/PR rates will also be computed for evaluable patients only. A detailed response status (complete response, partial response, stable, and progressive disease) will be summarized overall for the intent-to-treat and the evaluable populations. All responses, regardless of the duration, will be included in the above analyses.

Additional secondary efficacy endpoints are time to disease progression and survival. These will be analyzed after the 2- and 4-year data from all patients have been collected. Time to progression is defined as date of progression/death/last contact - date treatment started. Survival is defined as date of death/last contact - date treatment started. If the specified event (death/progression) did not occur during the follow-up period, the above variables will be censored at the last contact date.

Descriptive statistics such as median, minimum, and maximum values will be used to summarize the time to event data. Kaplan-Meier estimates will be used to present the time to progression data. Correlation between response to treatment and immunological parameter gathered from the correlative studies will be performed by Cox regression analysis.

A descriptive analysis of the correlative studies will be performed to define antibody-dependent cell-mediated cytotoxicty (ADCC) pre or post-pegfilgrastim/rituximab and CD20 cell levels. The study parameters will be summarized with simple summary statistics, including means, medians, ranges, and standard deviations. All data summaries based on these correlative studies will be descriptive and exploratory in nature, with the goal of developing further questions regarding the modulation of therapy, or regarding reasons for efficacy or lack of efficacy.

Supportive Efficacy. The supportive analysis will include 2-and 4-year survival estimates, overall performance on study (weight loss, change in Karnofsky Score), and exploratory analysis of the effects of baseline characteristics on response rates, time to progression, and survival.

9.5 Determination of Sample Size

Twenty patients will be used for the first end point which is safety evaluation and the primary analysis. The standard error of the percentage of patients who did not experience a DLT will be at most 11 percentage points. With a sample size of 40 evaluable patients, the precision of the estimate of the ORR rate, which is reflected by the width of the 95% confidence interval, will be at most 31 percentage points.

10.0 STUDY DRUG INFORMATION AND ACCOUNTABILITY

10.1 Rituximab (Rituxan) – commercially available drug

10.1.1 Formulation and Storage

Provided in 100 mg (10 mL) or 500 mg (50 mL) vials at a concentration of 10 mg/mL.

The rituximab drug product is formulated in 25 mm sodium citrate, 150 mm sodium chloride, 0.07% polysorbate 80 and buffered to a pH of 6.5 using either hydrochloric acid or sodium hydroxide.

Rituximab must be diluted in normal saline to achieve a final concentration of between 1 mg/mL and 4 mg/mL. Care should be taken during drug dilution to avoid shaking, as this can lead to aggregation of precipitation of the antibody. Rituximab for clinical use should be stored in a secure

refrigerator at 2˚C to 8˚C. Rituximab must be stored, prepared, and administered according to the directions in the package insert.

10.1.2 Adverse reactions and Warnings

For further details on rituximab including adverse reactions and warning, see appendix A.

WARNINGS

Fatal Infusion Reactions: Deaths within 24 hours of RITUXAN infusion have been reported. These fatal reactions followed an infusion reaction complex, which included hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, or cardiogenic shock. Approximately 80% of fatal infusion reactions occurred in association with the first infusion. (See WARNINGS and ADVERSE REACTIONS.)

Patients who develop severe infusion reactions should have RITUXAN infusion discontinued and receive medical treatment.

Tumor Lysis Syndrome (TLS): Acute renal failure requiring dialysis with instances of fatal outcome has been reported in the setting of TLS following treatment of non-Hodgkin's lymphoma (NHL) patients with RITUXAN. (See WARNINGS.)

Severe Mucocutaneous Reactions: Severe mucocutaneous reactions, some with fatal outcome, have been reported in association with RITUXAN treatment. (See WARNINGS and ADVERSE REACTIONS.)

Severe Infusion Reactions (See BOXED WARNINGS, ADVERSE REACTIONS and Hypersensitivity Reactions)

RITUXAN has caused severe infusion reactions. In some cases, these reactions were fatal. These severe reactions typically occurred during the first infusion with time to onset of 30 to 120 minutes. Signs and symptoms of severe infusion reactions may include hypotension, angioedema, hypoxia or bronchospasm, and may require interruption of RITUXAN administration. The most severe manifestations and sequelae include pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, and cardiogenic shock. In the reported cases, the following factors were more frequently associated with fatal outcomes: female gender, pulmonary infiltrates, and chronic lymphocytic leukemia or mantle cell lymphoma.

Management of severe infusion reactions: The RITUXAN infusion should be interrupted for severe reactions and supportive care measures instituted as medically indicated (e.g., intravenous fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen). In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Patients requiring close monitoring during first and all subsequent infusions include those with pre-existing cardiac and pulmonary conditions, those with prior clinically significant cardiopulmonary adverse events and those with high numbers of circulating malignant cells ($\geq$ 25,000/mm^3) with or without evidence of high tumor burden.

Tumor Lysis Syndrome [TLS] (See BOXED WARNINGS and ADVERSE REACTIONS) Rapid reduction in tumor volume followed by acute renal failure, hyperkalemia, hypocalcemia, hyperuricemia, or hyperphosphatasemia, have been reported within 12 to 24 hours after the first RITUXAN infusion. Rare instances of fatal outcome have been reported in the setting of TLS following treatment with RITUXAN. The risks of TLS appear to be greater in patients with high numbers of circulating malignant cells ($\geq$ 25,000/mm^3) or high tumor burden. Prophylaxis for TLS should be considered for patients at high risk. Correction of electrolyte abnormalities, monitoring of renal function and fluid balance, and administration of supportive care, including dialysis, should be initiated as indicated. Following complete resolution of the complications of TLS, RITUXAN has been tolerated when re-administered in conjunction with prophylactic therapy for TLS in a limited number of cases.

Hepatitis B Reactivation with Related Fulminant Hepatitis and Other Viral Infections Hepatitis B virus (HBV) reactivation with fulminant hepatitis, hepatic failure, and death has been reported in some patients with hematologic malignancies treated with RITUXAN. The majority of patients received RITUXAN in combination with chemotherapy. The median time to the diagnosis of hepatitis was approximately 4 months after the initiation of RITUXAN and approximately one month after the last dose.

Persons at high risk of HBV infection should be screened before initiation of RITUXAN. Carriers of hepatitis B should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis during and for up to several months following RITUXAN therapy. In patients who develop viral hepatitis, RITUXAN and any concomitant chemotherapy should be discontinued and appropriate treatment including antiviral therapy initiated. There are insufficient data regarding the safety of resuming RITUXAN therapy in patients who develop hepatitis subsequent to HBV reactivation.

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

Hypersensitivity Reactions RITUXAN has been associated with hypersensitivity reactions (non-IgE-mediated reactions) which may

respond to adjustments in the infusion rate and in medical management. Hypotension, bronchospasm, and angioedema have occurred in association with RITUXAN infusion (see Severe Infusion Reactions). RITUXAN infusion should be interrupted for severe hypersensitivity reactions and can be resumed at a 50% reduction in rate (e.g., from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Treatment of these symptoms with diphenhydramine and acetaminophen is recommended; additional treatment with bronchodilators or IV saline may be indicated. In most cases, patients who have experienced non-life-threatening hypersensitivity reactions have been able to complete the full course of therapy. (See DOSAGE and ADMINISTRATION.) Medications for the treatment of hypersensitivity reactions, e.g., epinephrine, antihistamines and corticosteroids, should be available for immediate use in the event of a reaction during administration.

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

Renal (See BOXED WARNINGS: Tumor Lysis Syndrome [TLS] and ADVERSE REACTIONS) RITUXAN administration has been associated with severe renal toxicity including acute renal failure requiring dialysis and in some cases, has led to a fatal outcome. Renal toxicity has occurred in patients with high numbers of circulating malignant cells (>25,000/mm^3) or high tumor burden who experience tumor lysis syndrome (see Tumor Lysis Syndrome) and in patients administered concomitant cisplatin therapy during clinical trials. The combination of cisplatin and RITUXAN is not an approved treatment regimen. If this combination is used in clinical trials *extreme caution* should be exercised; patients should be monitored closely for signs of renal failure. Discontinuation of RITUXAN should be considered for those with rising serum creatinine or oliguria.

Severe Mucocutaneous Reactions (See BOXED WARNINGS) Mucocutaneous reactions, some with fatal outcome, have been reported in patients treated with RITUXAN. These reports include paraneoplastic pemphigus (an uncommon disorder which is a manifestation of the patient's underlying malignancy),[18] Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. The onset of the reaction in the reported cases has varied from 1 to 13 weeks following RITUXAN exposure. Patients experiencing a severe mucocutaneous reaction should not receive any further infusions and seek prompt medical evaluation. Skin biopsy may help to distinguish among different mucocutaneous reactions and guide subsequent treatment. The

safety of readministration of RITUXAN to patients with any of these mucocutaneous reactions has not been determined.

Bowel Obstruction and Perforation Abdominal pain, bowel obstruction and perforation, in some cases leading to death, were observed in patients receiving RITUXAN in combination with chemotherapy for DLBCL. In post-marketing reports, which include both patients with low-grade or follicular NHL and DLBCL, the mean time to onset of symptoms was 6 days (range 1–77) in patients with documented gastro-intestinal perforation. Complaints of abdominal pain, especially early in the course of treatment, should prompt a thorough diagnostic evaluation and appropriate treatment.

Adverse Reactions
Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to drug use and for approximating rates.

The overall safety database for RITUXAN is based on clinical trial data from 1283 patients with NHL, who received RITUXAN either as a single agent or in combination with chemotherapy. Additional safety information was obtained from post-marketing safety surveillance. The most common adverse reactions were infusion reactions (see INFUSION REACTIONS below).

The following serious adverse reactions, some with fatal outcomes, have been reported in patients treated with RITUXAN (see BOXED WARNINGS and WARNINGS): severe or fatal infusion reactions, tumor lysis syndrome, severe mucocutaneous reactions, hepatitis B reactivation with fulminant hepatitis, other viral infections, hypersensitivity reactions, cardiac arrhythmias, renal toxicity, bowel obstruction and perforation.
Except as noted, adverse events described below occurred in the setting of relapsed or refractory, low-grade or follicular, CD20-positive, B-cell, NHL and are based on 356 patients treated in nonrandomized, single-arm studies of RITUXAN administered as a single agent. Most patients received RITUXAN 375 mg/m^2 weekly for 4 doses.

Infusion Reactions (See BOXED WARNINGS and WARNINGS)
Mild to moderate infusion reactions consisting of fever and chills/rigors occurred in the majority of patients during the first RITUXAN infusion. Other frequent infusion reaction symptoms included nausea, pruritus, angioedema, asthenia, hypotension, headache, bronchospasm, throat irritation, rhinitis, urticaria, rash, vomiting, myalgia, dizziness, and

hypertension. These reactions generally occurred within 30 to 120 minutes of beginning the first infusion, and resolved with slowing or interruption of the RITUXAN infusion and with supportive care (diphenhydramine, acetaminophen, IV saline, and vasopressors). The incidence of infusion reactions was highest during the first infusion (77%) and decreased with each subsequent infusion (30% with fourth infusion and 14% with eighth infusion). Injection site pain was reported in less than 5% of patients.

Infectious Events (See WARNINGS: Hepatitis B Reactivation with Related Fulminant Hepatitis and Other Viral Infections)
RITUXAN induced B-cell depletion in 70% to 80% of patients and was associated with decreased serum immunoglobulins in a minority of patients; the lymphopenia lasted a median of 14 days (range, 1 to 588 days). Infectious events occurred in 31% of patients: 19% of patients had bacterial infections, 10% had viral infections, 1% had fungal infections, and 6% were unknown infections. Incidence is not additive because a single patient may have had more than one type of infection. Serious infectious events (Grade 3 or 4), including sepsis, occurred in 2% of patients.

Hematologic Events
Grade 3 and 4 cytopenias were reported in 48% of patients treated with RITUXAN; these include: lymphopenia (40%), neutropenia (6%), leukopenia (4%), anemia (3%), and thrombocytopenia (2%). The median duration of lymphopenia was 14 days (range, 1 to 588 days) and of neutropenia was 13 days (range, 2 to 116 days). A single occurrence of transient aplastic anemia (pure red cell aplasia) and two occurrences of hemolytic anemia following RITUXAN therapy were reported.

Pulmonary Events
135 patients (38%) experienced pulmonary events in clinical trials. The most common respiratory system adverse events experienced were increased cough, rhinitis, bronchospasm, dyspnea, and sinusitis. In both clinical studies and post-marketing surveillance, there have been a limited number of reports of bronchiolitis obliterans presenting up to 6 months post-RITUXAN infusion and a limited number of reports of pneumonitis (including interstitial pneumonitis) presenting up to 3 months post-RITUXAN infusion, some of which resulted in fatal outcomes. The safety of resumption or continued administration of RITUXAN in patients with pneumonitis or bronchiolitis obliterans is unknown.

Immunogenicity
The observed incidence of antibody positivity in an assay is highly dependent on the sensitivity and specificity of the assay and may be influenced by several factors including sample handling, concomitant medications, and underlying disease. For these reasons, comparison of

the incidence of antibodies to RITUXAN with the incidence of antibodies to other products may be misleading.

In clinical studies of patients with low-grade or follicular NHL receiving single-agent RITUXAN, human antichimeric antibody (HACA) was detected in 4 of 356 (1.1%) patients and 3 had an objective clinical response. These data reflect the percentage of patients whose test results were considered positive for antibodies to RITUXAN using an enzyme-linked immunosorbant assay (limit of detection = 7 ng/mL).

Single Agent RITUXAN for Relapsed or Refractory, Low-Grade or Follicular, CD20-Positive, B-Cell, NHL

Study subjects ranged from 22 to 81 years of age. Sixty percent were male; 93% were Caucasian, 1% were African American, 2% were Hispanic, 2% were Asian, and 2% were from other racial groups. Table 4 lists the most common, as well as Grade 3 and 4, adverse events observed.

Table 4
Incidence of Adverse Events in ≥ 5% of Patients with Relapsed or Refractory, Low-Grade or Follicular NHL, Receiving Single-agent RITUXAN (N=356)[a,b]

	All Grades (%)	Grade 3 and 4 (%)
Any Adverse Events	**99**	**57**
Body as a Whole	**86**	**10**
Fever	53	1
Chills	33	3
Infection	31	4
Asthenia	26	1
Headache	19	1
Abdominal Pain	14	1
Pain	12	1
Back Pain	10	1
Throat Irritation	9	0
Flushing	5	0
Cardiovascular System	**25**	**3**
Hypotension	10	1
Hypertension	6	1
Digestive System	**37**	**2**
Nausea	23	1

Diarrhea	10	1
Vomiting	10	1
Hemic and Lymphatic System	**67**	**48**
Lymphopenia	48	40
Leukopenia	14	4
Neutropenia	14	6
Thrombocytopenia	12	2
Anemia	8	3
Metabolic and Nutritional Disorders	**38**	**3**
Angioedema	11	1
Hyperglycemia	9	1
Peripheral Edema	8	0
LDH Increase	7	0
Musculoskeletal System	**26**	**3**
Myalgia	10	1
Arthralgia	10	1
Nervous System	**32**	**1**
Dizziness	10	1
Anxiety	5	1
Respiratory System	**38**	**4**
Increased Cough	13	1
Rhinitis	12	1
Bronchospasm	8	1
Dyspnea	7	1
Sinusitis	6	0
Skin and Appendages	**44**	**2**
Night Sweats	15	1
Rash	15	1
Pruritus	14	1
Urticaria	8	1

[a] Adverse Events observed up to 12 months following RITUXAN.

[b] Adverse Events graded for severity by NCI-CTC criteria.[19]

Risk Factors Associated with Increased Rates of Adverse Events

Administration of RITUXAN weekly for 8 doses resulted in higher rates of Grade 3 and 4 adverse events[14] overall (70%) compared with administration weekly for 4 doses (57%). The incidence of Grade 3 or 4 adverse events was similar in patients retreated with RITUXAN compared with initial treatment (58% and 57%, respectively). The incidence of the following clinically significant adverse events was higher in patients with bulky disease (lesions ≥ 10 cm) (N = 39) versus patients with lesions < 10 cm (N = 195): abdominal pain, anemia, dyspnea, hypotension, and neutropenia.

10.2 Pegfilgrastim Drug Information – investigational drug provided

10.2.1 Packaging and Formulation

Pegfilgrastim will be manufactured and packaged by Amgen Inc. and distributed using Amgen clinical trial drug distribution procedures.

Pegfilgrastim is supplied as a preservative-free solution containing 6 mg (0.6 mL) of pegfilgrastim (10 mg/mL) in a single-dose syringe with a 27 gauge, 1/2 inch needle with an UltraSafe® Needle Guard.

Neulasta® is supplied in 0.6 mL prefilled syringes for subcutaneous injection. Each syringe contains 6 mg pegfilgrastim (based on protein weight), in a sterile, clear, colorless, preservative-free solution (pH 4.0) containing acetate (0.35 mg), sorbitol (30.0 mg), polysorbate 20 (0.02 mg), and sodium (0.02 mg) in water for injection, USP.

10.2.2 Labeling

Drug labeling will comply with the requirements of the U.S. FDA.

EACH PREFILLED SYRINGE OF PEGFILGRASTIM WILL BE LABELED WITH THE FOLLOWING:

pegfilgrastim 10 mg/mL
0.6 mL s.c.
CAUTION: New drug – Limited
by Federal Law to
Investigational Use
Amgen Inc. 2°C - 8°C

10.2.3. Supply and Return of Drug

At study initiation and as needed thereafter, pegfilgrastim will be shipped to the Pharmacist at the investigator's institution, who will check the amount and condition of the drug and enter these data into the Proof of Receipt form and Investigational Product Accountability Record. The Proof of Receipt form should then be faxed to Amgen and the original retained at the center. At the end of the study, or as directed, all pegfilgrastim unused prefilled syringes will be destroyed per the site's standard operating procedures.

10.2.4. Drug Accountability

An Accountability Record for the clinical trial product, pegfilgrastim, must be kept current, and should contain:

- the dates and quantities of drug received from Amgen, Inc
- subject's identification (subject number and initials and lot number)
- date and quantity of drug dispensed (and remaining)
- the initials of the dispenser, and
- date and quantity of drug returned to the investigator/pharmacy, if appropriate

Pegfilgrastim prefilled syringes that have been used will be discarded per institution policy. At the end of the study, the Final Product Reconciliation Statement must be completed and provided to Amgen. These inventories must be made available for inspection by an authorized Amgen representative or designee and regulatory agency inspectors. The investigator is responsible for the accountability of all used and unused trial supplies.

10.2.5. Storage Conditions and Stability

Pegfilgrastim should be stored refrigerated at 2° to 8°C (36° to 46°F); syringes should be kept in their carton to protect from light until time of use. Shaking should be avoided. Before injection, pegfilgrastim may be allowed to reach room temperature for a maximum of 48 hours but should be protected from light. Pegfilgrastim left at room temperature for more than 48 hours should be discarded. Freezing should be avoided; however, if accidentally frozen, pegfilgrastim should be allowed to thaw in the refrigerator before administration. If frozen a second time, pegfilgrastim should be discarded. Pegfilgrastim should be visually inspected for discoloration and particulate matter before administration. Pegfilgrastim should not be administered if discoloration or particulates are observed.

Since pegfilgrastim provided for clinical trials contains no preservatives, prefilled syringes are designed for single use only. Contact your Amgen representative, or their designee, if storage conditions fall out of the specified range. Before investigational product that has been exposed to storage conditions out of the specified range can be used, Amgen's Stability Department must issue a memo. This memo must be sent to and maintained at the site.

Records of the actual storage conditions during the period of the study must be maintained (eg, records of the date and time and initials of person checking, and the daily temperatures of the refrigerator used for storage of

investigational product, continuous temperature recordings, or regularly maintained temperature alarm systems).

10.2.6. Preparation and Administration

No preparation is required for administration of pegfilgrastim. Each subject will receive afixed dose of 6 mg of pegfilgrastim. The entire contents of the 0.6 mL prefilled syringe should be administered irrespective of the subject's actual weight.

For method of administration please see patient package insert.

10.2.7. Adverse Reactions

In a placebo-controlled trial, bone pain occurred at a higher incidence in pegfilgrastim-treated patients as compared to placebo-treated patients (Pegfilgrastim, n = 467; Placebo, n = 461). The incidence of other commonly reported adverse events were similar in the pegfilgrastim- and placebo-treated patients, and were consistent with the underlying cancer diagnosis and its treatment with chemotherapy. Those adverse events occurred at rates between 48% and 10% in the pegfilgrastim treated patients and included: alopecia, bone pain, diarrhea, pyrexia (not including febrile neutropenia), myalgia, headache, arthralgia, vomiting, asthenia, edema peripheral, and constipation.

In the active controlled studies, common adverse events occurred at similar rates and severities in both treatment arms (Pegfilgrastim, n = 465; Filgrastim, n = 331). These adverse experiences occurred at rates between 72% and 15% and included: nausea, fatigue, alopecia, diarrhea, vomiting, constipation, fever, anorexia, skeletal pain, headache, taste perversion, dyspepsia, myalgia, insomnia, abdominal pain, arthralgia, generalized weakness, peripheral edema, dizziness, granulocytopenia, stomatitis, mucositis, and neutropenic fever.

Bone Pain

In the placebo-controlled study, the incidence of bone pain was 57% in Neulasta®-treated patients compared to 50% in placebo-treated patients. Bone pain was generally reported to be of mild-to-moderate severity.

Among patients experiencing bone pain, approximately 37% of Neulasta®- and 31% of placebo-treated patients utilized non-narcotic analgesics and 10% of Neulasta®- and 9% of placebo-treated patients utilized narcotic analgesics.

In the active-controlled studies, the use of non-narcotic and narcotic analgesics in association with bone pain was similar between Neulasta®- and Filgrastim-treated patients. No patient withdrew from study due to bone pain.

Laboratory Abnormalities
In clinical studies, leukocytosis (WBC counts > 100 x 10^9/L) was observed in less than 1% of 932 patients with non-myeloid malignancies receiving Neulasta®. Leukocytosis was not associated with any adverse effects.
In the placebo-controlled study, reversible elevations in LDH, alkaline phosphatase, and uric acid that did not require treatment occurred at similar rates in Neulasta®- and placebo-treated patients.

Overdosage
The maximum amount of Neulasta® that can be safely administered in single or multiple doses has not been determined. Single doses of 300 mcg/kg have been administered SC to 8 normal volunteers and 3 patients with non-small cell lung cancer without serious adverse effects. These subjects experienced a mean maximum ANC of 55 x 10^9/L, with a corresponding mean maximum WBC of 67 x 10^9/L. The absolute maximum ANC observed was 96 x 10^9/L with a corresponding absolute maximum WBC observed of 120 x 10^9/L. The duration of leukocytosis ranged from 6 to 13 days. Leukapheresis should be considered in the management of symptomatic individuals.

Toxicity/Warnings
Pegfilgrastim is contraindicated inpatients with known hypersensitivity to *E coli*-derived proteins, pegfilgrastim, Filgrastim, or any other component of the product.

Rare cases of splenic rupture have been reported following the administration of Neulasta®. Splenic rupture, in some cases resulting in death, has also been associated with filgrastim, the parent compound of pegfilgrastim. Patients receiving pegfilgrastim who report left upper abdominal and/or shoulder tip pain should be evaluated for an enlarged spleen or splenic rupture.

Adult respiratory distress syndrome (ARDS) has been reported in neutropenic patients with sepsis receiving Filgrastim, the parent compound of pegfilgrastim, and is postulated to be secondary to an influx of neutrophils to sites of inflammation in the lungs. Neutropenic patients receiving pegfilgrastim who develop fever, lung infiltrates, or respiratory distress should be evaluated for the possibility of ARDS. In the event that ARDS occurs, pegfilgrastim should be discontinued and/or withheld until resolution of ARDS and patients should receive appropriate medical management for this condition. Moreover It is unknown if the risk of acute respiratory distress syndrome with Rituximab will be higher with the use of pegfilgrastim.

Allergic reactions to pegfilgrastim, including anaphylaxis, skin rash, and urticaria, have been reported in post marketing experience. The majority of reported events occurred upon initial exposure. In some cases, symptoms recurred with rechallenge, suggesting a causal relationship. In rare cases, allergic reactions including anaphylaxis, recurred within days after initial anti-allergic treatment was discontinued. If a serious allergic reaction occurs, appropriate therapy should be administered, with close patient follow-up over several days. Pegfilgrastim should be permanently discontinued in patients with serious allergic reactions.

Severe sickle cell crises have been associated with the use of Neulasta® in patients with sickle cell disease. Severe sickle cell crises, in some cases resulting in death, have also been associated with Filgrastim, the parent compound of pegfilgrastim. Only physicians qualified by specialized training or experience in the treatment of patients with sickle cell disease should prescribe Neulasta® for such patients, and only after careful consideration of the potential risks and benefits.

Pregnancy and Lactation
There are no adequate and well-controlled studies in pregnant women. The risks of the study drug to an unborn or newborn child are not known. In addition, it is not known whether pegfilgrastim is secreted in human milk. Therefore, pregnant or nursing mothers may not take part in this study.

Drug Interactions
No formal drug interaction studies between Neulasta® and other drugs have been performed. Drugs such as lithium may potentiate the release of neutrophils; patients receiving lithium and Neulasta® should have more frequent monitoring of neutrophil counts.

11.0 ETHICAL CONSIDERATIONS

11.1 Investigator Requirements

11.1.1 Study Initiation

Before the start of this study, the following documents must be on file with Amgen or an Amgen representative:

- Original U.S. FDA Form 1572 (for all studies conducted under U.S. Investigational New Drug [IND] regulations), signed by all Principal Investigators.
    - The names of any sub investigators must appear on this form. Investigators must also complete all regulatory documentation as required by local and national regulations.

- Current curricula vitae of the Principal Investigator and all sub investigators.
- Institutional Review Board/Ethical Committee (IRB/EC) membership list and/or Department of Health and Human Services number.
- written documentation of IRB/EC approval of protocol (identified by Amgen protocol number or title and date of approval) and informed consent document (identified by Amgen protocol number or title and date of approval).
- a copy of the IRB/EC approved informed consent document.
- written documentation of IRB/EC review and approval of any advertising materials to be used for study recruitment, if applicable.
- Current laboratory certification of the laboratory performing the analysis (if other than a Genentech approved central laboratory), as well as current normal laboratory ranges for all laboratory tests.
- A signed Clinical Research Agreement.
- Certified translations of IRB/EC approval letters, pertinent correspondence, and approved informed consent document (when applicable).
- A signed and dated protocol signature page.

11.1.2 Study Completion

The following data and materials are required before a study can be considered complete or terminated:

- Laboratory findings, clinical data, and all special test result from screening through the end of the study follow-up period.
- All Electronic Data Capture Reports properly maintained by study personnel and the investigator.
- Completed Drug Accountability Records (Investigational New Drug Retrieval Record [INDRR-1], Drug Inventory Log, and Inventory of Returned Clinical Material forms).
- Copies of protocol amendments and IRB/EC approval/notification, if appropriate.
- A summary of the study prepared by the Principal Investigator (will accept IRB summary close letter).

11.2 Informed Consent

An informed consent document for this proposed study has been written .

The patient or the patient's legal guardian before his or her participation in the study must sign the informed consent document. A copy of the informed consent document must be provided to the patient or the patient's legal guardian. If applicable, it will be translated to the language understood by the patient.

Signed consent forms must remain in each patient's study file and must be available for verification by study monitors at any time.

11.3 Institutional Review Board or Ethics Committee Approval

This protocol, the informed consent document, and relevant supporting information must be submitted to the IRB/EC for review and must be approved before the study is initiated.

The Principal Investigator is responsible for keeping the IRB/EC appraised of the progress of the study and of any changes made to the protocol as deemed appropriate, but in any case at least once a year. The Principal Investigator must also keep the IRB/EC informed of any significant adverse events.

12.0 DATA QUALITY ASSURANCE

Accurate, consistent, and reliable data will be ensured though the use of standard practices and procedures.

13.0 DISCLOSURE OF DATA

Patient medical information obtained by this study is confidential, and disclosure to third parties other than those noted below is prohibited.

Upon the patient's permission, medical information may be given to his or her personal physician or other appropriate medical personnel responsible for his or her welfare.

Data generated by this study must be available for inspection upon request by representatives of the U.S. FDA, national and local health authorities, and the IRB/EC for each study site, if appropriate.

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Appendix A:
[Neulasta®] (pegfilgrastim) Prescribing Information

DESCRIPTION

Neulasta® (pegfilgrastim) is a covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol. Filgrastim is a water-soluble 175 amino acid protein with a molecular weight of approximately 19 kilodaltons (kD). Filgrastim is obtained from the bacterial fermentation of a strain of *Escherichia coli* transformed with a genetically engineered plasmid containing the human G-CSF gene. To produce pegfilgrastim, a 20 kD monomethoxypolyethylene glycol molecule is covalently bound to the N-terminal methionyl residue of Filgrastim. The average molecular weight of pegfilgrastim is approximately 39 kD.

Neulasta® is supplied in 0.6 mL prefilled syringes for subcutaneous injection. Each syringe contains 6 mg pegfilgrastim (based on protein weight), in a sterile, clear, colorless, preservative-free solution (pH 4.0) containing acetate (0.35 mg), sorbitol (30.0 mg), polysorbate 20 (0.02 mg), and sodium (0.02 mg) in water for injection, USP.

CLINICAL PHARMACOLOGY

Both Filgrastim and pegfilgrastim are Colony Stimulating Factors that act on hematopoietic cells by binding to specific cell surface receptors thereby stimulating proliferation, differentiation, commitment, and end cell functional activation.[1,2] Studies on cellular proliferation, receptor binding, and neutrophil function demonstrate that Filgrastim and pegfilgrastim have the same mechanism of action. Pegfilgrastim has reduced renal clearance and prolonged persistence in vivo as compared to Filgrastim.

Pharmacokinetics

The pharmacokinetics and pharmacodynamics of Neulasta® were studied in 379 patients with cancer. The pharmacokinetics of Neulasta® were nonlinear in cancer patients and clearance decreased with increases in dose. Neutrophil receptor binding is an important component of the clearance of Neulasta®, and serum clearance is directly related to the number of neutrophils. For example, the concentration of Neulasta® declined rapidly at the onset of neutrophil recovery that followed myelosuppressive chemotherapy. In addition to numbers of neutrophils, body weight appeared to be a factor. Patients with higher body weights experienced higher systemic exposure to Neulasta® after receiving a dose normalized for body weight. A large variability in the pharmacokinetics of Neulasta® was observed in cancer patients. The half-life of Neulasta® ranged from 15 to 80 hours after subcutaneous injection.

Special Populations

No gender-related differences were observed in the pharmacokinetics of Neulasta®, and no differences were observed in the pharmacokinetics of geriatric patients (≥ 65 years of age)

compared to younger patients (< 65 years of age) (see PRECAUTIONS, Geriatric Use). In a study of 30 patients with varying degrees of renal dysfunction including end-stage renal disease, renal dysfunction had no effect on the pharmacokinetics of pegfilgrastim; thus, dose adjustment in patients with renal dysfunction is not necessary. The pharmacokinetic profile in pediatric populations or in patients with hepatic insufficiency has not been assessed.

CLINICAL STUDIES

Neulasta® was evaluated in three randomized, double-blind, controlled studies. Studies 1 and 2 were active-controlled studies that employed doxorubicin 60 mg/m^2 and docetaxel 75 mg/m^2 administered every 21 days for up to 4 cycles for the treatment of metastatic breast cancer. Study 1 investigated the utility of a fixed dose of Neulasta®. Study 2 employed a weight-adjusted dose. In the absence of growth factor support, similar chemotherapy regimens have been reported to result in a 100% incidence of severe neutropenia (absolute neutrophil count [ANC] < 0.5 x 10^9/L) with a mean duration of 5-7 days, and a 30%-40% incidence of febrile neutropenia. Based on the correlation between the duration of severe neutropenia and the incidence of febrile neutropenia found in studies with Filgrastim, duration of severe neutropenia was chosen as the primary endpoint in both studies, and the efficacy of Neulasta® was demonstrated by establishing comparability to Filgrastim-treated patients in the mean days of severe neutropenia.

In Study 1, 157 patients were randomized to receive a single subcutaneous injection of Neulasta® 6 mg on day 2 of each chemotherapy cycle or daily subcutaneous Filgrastim 5 mcg/kg/day beginning on day 2 of each chemotherapy cycle. In Study 2, 310 patients were randomized to receive a single subcutaneous injection of Neulasta® 100 mcg/kg on day 2 or daily subcutaneous Filgrastim 5 mcg/kg/day beginning on day 2 of each chemotherapy cycle.

Both studies met the primary objective of demonstrating that the mean days of severe neutropenia of Neulasta®-treated patients did not exceed that of Filgrastim-treated patients by more than one day in cycle 1 of chemotherapy (see Table 1). The rates of febrile neutropenia in the two studies were comparable for Neulasta® and Filgrastim (in the range of 10% to 20%). Other secondary endpoints included days of severe neutropenia in cycles 2-4, the depth of ANC nadir in cycles 1-4, and the time to ANC recovery after nadir. In both studies, the results for the secondary endpoints were similar between the two treatment groups.

Table 1. Mean Days of Severe Neutropenia (in Cycle 1)

Study	Mean days of severe neutropenia		Difference in means (95% CI)
	Neulasta®	Filgrastim (5 mcg/kg/day)	
Study 1 n = 157	1.8	1.6	0.2 (-0.2, 0.6)
Study 2 n = 310	1.7	1.6	0.1 (-0.2, 0.4)
[a] Study 1 dose = 6 mg x 1; study 2 dose = 100 mcg/kg x 1			

Study 3 was a randomized, double-blind, placebo-controlled study that employed docetaxel 100 mg/m^2 administered every 21 days for up to 4 cycles for the treatment of metastatic or non-metastatic breast cancer. In this study, 928 patients were randomized to receive a single subcutaneous injection of Neulasta® 6 mg or placebo on day 2 of each chemotherapy cycle. Study 3 met the primary objective of demonstrating that the incidence of febrile neutropenia (defined as temperature ≥ 38.2°C and ANC ≤ 0.5 x10^9/L) was lower for Neulasta®-treated patients as compared to placebo-treated patients (1% versus 17%, p < 0.001). The incidence of hospitalizations (1% versus 14%) and IV anti-infective use (2% versus 10%) for the treatment of febrile neutropenia were also lower in the Neulasta®-treated patients compared with the placebo-treated patients.

INDICATIONS AND USAGE

Neulasta® is indicated to decrease the incidence of infection, as manifested by febrile neutropenia, in patients with non-myeloid malignancies receiving myelosuppressive anti-cancer drugs associated with a clinically significant incidence of febrile neutropenia (See CLINICAL STUDIES).

CONTRAINDICATIONS

Neulasta® is contraindicated in patients with known hypersensitivity to E coli-derived proteins, pegfilgrastim, Filgrastim, or any other component of the product.

WARNINGS

General

The safety and efficacy of Neulasta® for peripheral blood progenitor cell (PBPC) mobilization has not been evaluated in adequate and well-controlled studies. Neulasta® should not be used for PBPC mobilization.

Splenic Rupture

Rare cases of splenic rupture have been reported following the administration of neulasta®. Splenic rupture, in some cases resulting in death, has also been associated with filgrastim, the parent compound of neulasta®. Patients receiving neulasta® who report left upper abdominal and/or shoulder tip pain should be evaluated for an enlarged spleen or splenic rupture.

Adult Respiratory Distress Syndrome (ARDS)

Adult respiratory distress syndrome (ARDS) has been reported in neutropenic patients with sepsis receiving Filgrastim, the parent compound of Neulasta®, and is postulated to be secondary to an influx of neutrophils to sites of inflammation in the lungs. Neutropenic patients receiving Neulasta®

who develop fever, lung infiltrates, or respiratory distress should be evaluated for the possibility of ARDS. In the event that ARDS occurs, Neulasta® should be discontinued and/or withheld until resolution of ARDS and patients should receive appropriate medical management for this condition.

Allergic Reactions

Allergic reactions to Neulasta®, including anaphylaxis, skin rash, and urticaria, have been reported in post marketing experience. The majority of reported events occurred upon initial exposure. In some cases, symptoms recurred with rechallenge, suggesting a causal relationship. In rare cases, allergic reactions including anaphylaxis, recurred within days after initial anti-allergic treatment was discontinued. If a serious allergic reaction occurs, appropriate therapy should be administered, with close patient follow-up over several days. Neulasta® should be permanently discontinued in patients with serious allergic reactions.

Sickle Cell Disease

Severe sickle cell crises have been associated with the use of Neulasta® in patients with sickle cell disease. Severe sickle cell crises, in some cases resulting in death, have also been associated with Filgrastim, the parent compound of pegfilgrastim. Only physicians qualified by specialized training or experience in the treatment of patients with sickle cell disease should prescribe Neulasta® for such patients, and only after careful consideration of the potential risks and benefits.

PRECAUTIONS

General

Use With Chemotherapy and/or Radiation Therapy

Neulasta® should not be administered in the period between 14 days before and 24 hours after administration of cytotoxic chemotherapy (see DOSAGE AND ADMINISTRATION) because of the potential for an increase in sensitivity of rapidly dividing myeloid cells to cytotoxic chemotherapy.

The use of Neulasta® has not been studied in patients receiving chemotherapy associated with delayed myelosuppression (eg, nitrosoureas, mitomycin C).

The administration of Neulasta® concomitantly with 5-fluorouracil or other antimetabolites has not been evaluated in patients. Administration of pegfilgrastim at 0, 1, and 3 days before 5-fluorouracil resulted in increased mortality in mice; administration of pegfilgrastim 24 hours after 5-fluorouracil did not adversely affect survival.

The use of Neulasta® has not been studied in patients receiving radiation therapy.

Potential Effect on Malignant Cells

Pegfilgrastim is a growth factor that primarily stimulates neutrophils and neutrophil precursors; however, the G-CSF receptor through which pegfilgrastim and Filgrastim act has been found on tumor cell lines, including some myeloid, T-lymphoid, lung, head and neck, and bladder tumor cell

lines. The possibility that pegfilgrastim can act as a growth factor for any tumor type cannot be excluded. Use of Neulasta® in myeloid malignancies and myelodysplasia (MDS) has not been studied. In a randomized study comparing the effects of the parent compound of Neulasta®, Filgrastim, to placebo in patients undergoing remission induction and consolidation chemotherapy for acute myeloid leukemia, important differences in remission rate between the two arms were excluded. Disease-free survival and overall survival were comparable; however, the study was not designed to detect important differences in these endpoints.[3]

Information for Patients

Patients should be informed of the possible side effects of Neulasta®, and be instructed to report them to the prescribing physician. Patients should be informed of the signs and symptoms of allergic drug reactions and be advised of appropriate actions. Patients should be counseled on the importance of compliance with their Neulasta® treatment, including regular monitoring of blood counts.

If it is determined that a patient or caregiver can safely and effectively administer Neulasta® (pegfilgrastim) at home, appropriate instruction on the proper use of Neulasta® (pegfilgrastim) should be provided for patients and their caregivers, including careful review of the "Information for Patients and Caregivers" insert. Patients and caregivers should be cautioned against the reuse of needles, syringes, or drug product, and be thoroughly instructed in their proper disposal. A puncture-resistant container for the disposal of used syringes and needles should be available.

Laboratory Monitoring

To assess a patient's hematologic status and ability to tolerate myelosuppressive chemotherapy, a complete blood count and platelet count should be obtained before chemotherapy is administered. Regular monitoring of hematocrit value and platelet count is recommended.

Drug Interaction

No formal drug interaction studies between Neulasta® and other drugs have been performed. Drugs such as lithium may potentiate the release of neutrophils; patients receiving lithium and Neulasta® should have more frequent monitoring of neutrophil counts.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No mutagenesis studies were conducted with pegfilgrastim. The carcinogenic potential of pegfilgrastim has not been evaluated in long-term animal studies. In a toxicity study of 6 months duration in rats given once weekly subcutaneous injections of up to 1000 mcg/kg of pegfilgrastim (approximately 23-fold higher than the recommended human dose), no precancerous or cancerous lesions were noted.

When administered once weekly via subcutaneous injections to male and female rats at doses up to 1000 mcg/kg prior to, and during mating, reproductive performance, fertility, and sperm assessment parameters were not affected.

Pregnancy Category C

Pegfilgrastim has been shown to have adverse effects in pregnant rabbits when administered subcutaneously every other day during gestation at doses as low as 50 mcg/kg/dose (approximately 4-fold higher than the recommended human dose). Decreased maternal food consumption, accompanied by a decreased maternal body weight gain and decreased fetal body weights were observed at 50 to 1000 mcg/kg/dose. Pegfilgrastim doses of 200 and 250 mcg/kg/dose resulted in an increased incidence of abortions. Increased post-implantation loss due to early resorptions was observed at doses of 200 to 1000 mcg/kg/dose, and decreased numbers of live rabbit fetuses were observed at pegfilgrastim doses of 200 to 1000 mcg/kg/dose, given every other day.

Subcutaneous injections of pegfilgrastim of up to 1000 mcg/kg/dose every other day during the period of organogenesis in rats were not associated with an embryotoxic or fetotoxic outcome. However, an increased incidence (compared to historical controls) of wavy ribs was observed in rat fetuses at 1000 mcg/kg/dose every other day. Very low levels (< 0.5%) of pegfilgrastim crossed the placenta when administered subcutaneously to pregnant rats every other day during gestation.

Once weekly subcutaneous injections of pegfilgrastim to female rats from day 6 of gestation through day 18 of lactation at doses up to 1000 mcg/kg/dose did not result in any adverse maternal effects. There were no deleterious effects on the growth and development of the offspring and no adverse effects were found upon assessment of fertility indices. There are no adequate and well-controlled studies in pregnant women. Neulasta® should be used during pregnancy only if the potential benefit to the mother justifies the potential risk to the fetus.

Nursing Mothers

It is not known whether pegfilgrastim is excreted in human milk. Because many drugs are excreted in human milk, caution should be exercised when Neulasta® is administered to a nursing woman.

Pediatric Use

The safety and effectiveness of Neulasta® in pediatric patients have not been established. The 6 mg fixed dose single-use syringe formulation should not be used in infants, children, and smaller adolescents weighing less than 45 kg.

Geriatric Use

Of the 932 patients with cancer who received Neulasta® in clinical studies, 139 (15%) were age 65 and over, and 18 (2%) were age 75 and over. No overall differences in safety or effectiveness were observed between patients age 65 and older and younger patients.

ADVERSE REACTIONS

See WARNINGS sections regarding Splenic Rupture, ARDS, Allergic Reactions, and Sickle Cell Disease. Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of Neulasta® cannot be directly compared to rates in the clinical trials of other drugs and may not reflect the rates observed in practice. The adverse reaction

information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to Neulasta® use and for approximating rates.

The data described below reflect exposure to Neulasta® in 932 patients. Neulasta® was studied in placebo- and active-controlled trials (n = 467, and n = 465, respectively). The population encompassed an age range of 21 to 88 years. Ninety-two percent of patients were female. The ethnicity of the patients was as follows: 75% Caucasian, 18% Hispanic, 5% Black, and 1% Asian. Patients with solid tumors (breast [n = 823], lung and thoracic tumors [n = 53]) or lymphoma (n = 56) received Neulasta® after nonmyeloablative cytotoxic chemotherapy. Most patients received a single 100 mcg/kg (n = 259) or a single 6 mg (n = 546) dose per chemotherapy cycle over 4 cycles.

In the placebo-controlled trial, bone pain occurred at a higher incidence in Neulasta®-treated patients as compared to placebo-treated patients. The incidence of other commonly reported adverse events were similar in the Neulasta®- and placebo-treated patients, and were consistent with the underlying cancer diagnosis and its treatment with chemotherapy. The data in Table 2 reflect those adverse events occurring in at least 10% of patients treated with Neulasta® in the placebo-controlled study.

TABLE 2. Adverse Events Occurring in ≥ 10%[a] of Patients in The Placebo-Controlled Study.

Event	Neulasta® (n = 467)	Placebo (n = 461)
Alopecia	48%	47%
Bone Pain[b]	31%	26%
Diarrhea	29%	28%
Pyrexia (not including febrile neutropenia)	23%	22%
Myalgia	21%	18%
Headache	16%	14%
Arthralgia	16%	13%
Vomiting	13%	11%
Asthenia	13%	11%
Edema peripheral	12%	10%
Constipation	10%	6%

[a] Events occurring in ≥ 10% of Neulasta®-treated patients and at a higher incidence as compared to placebo-treated patients.

[b] Bone pain is limited to the specified adverse event term “bone pain.”

In the active controlled studies, common adverse events occurred at similar rates and severities in both treatment arms (Neulasta®, n = 465; Filgrastim, n = 331). These adverse experiences occurred at rates between 72% and 15% and included: nausea, fatigue, alopecia, diarrhea, vomiting, constipation, fever, anorexia, skeletal pain, headache, taste perversion, dyspepsia, myalgia, insomnia, abdominal pain, arthralgia, generalized weakness, peripheral edema, dizziness, granulocytopenia, stomatitis, mucositis, and neutropenic fever.

Bone Pain

The analysis of bone pain described below is based on a composite analysis using multiple, related, adverse event terms.

In the placebo-controlled study, the incidence of bone pain was 57% in Neulasta®-treated patients compared to 50% in placebo-treated patients. Bone pain was generally reported to be of mild-to-moderate severity.

Among patients experiencing bone pain, approximately 37% of Neulasta®- and 31% of placebo-treated patients utilized non-narcotic analgesics and 10% of Neulasta®- and 9% of placebo-treated patients utilized narcotic analgesics.

In the active-controlled studies, the use of non-narcotic and narcotic analgesics in association with bone pain was similar between Neulasta®- and Filgrastim-treated patients. No patient withdrew from study due to bone pain.

Laboratory Abnormalities

In clinical studies, leukocytosis (WBC counts > 100 x 10^9/L) was observed in less than 1% of 932 patients with non-myeloid malignancies receiving Neulasta®. Leukocytosis was not associated with any adverse effects.

In the placebo-controlled study, reversible elevations in LDH, alkaline phosphatase, and uric acid that did not require treatment occurred at similar rates in Neulasta®- and placebo-treated patients.

Immunogenicity

As with all therapeutic proteins, there is a potential for immunogenicity. The incidence of antibody development in patients receiving Neulasta® has not been adequately determined. While available data suggest that a small proportion of patients developed binding antibodies to Filgrastim or pegfilgrastim, the nature and specificity of these antibodies has not been adequately studied. No neutralizing antibodies have been detected using a cell-based bioassay in 46 patients who apparently developed binding antibodies. The detection of antibody formation is highly dependent on the sensitivity and specificity of the assay, and the observed incidence of antibody positivity in an assay may be influenced by several factors including sample handling, concomitant medications, and underlying disease. Therefore, comparison of the incidence of antibodies to Neulasta® with the incidence of antibodies to other products may be misleading.

Cytopenias resulting from an antibody response to exogenous growth factors have been reported on rare occasions in patients treated with other recombinant growth factors. There is a theoretical

possibility that an antibody directed against pegfilgrastim may cross-react with endogenous G-CSF, resulting in immune-mediated neutropenia, but this has not been observed in clinical studies.

OVERDOSAGE

The maximum amount of Neulasta® that can be safely administered in single or multiple doses has not been determined. Single subcutaneous doses of 300 mcg/kg have been administered to 8 healthy volunteers and 3 patients with non-small cell lung cancer without serious adverse effects. These patients experienced a mean maximum ANC of 55 x 10^9/L, with a corresponding mean maximum WBC of 67 x 10^9/L. The absolute maximum ANC observed was 96 x 10^9/L with a corresponding absolute maximum WBC observed of 120 x 10^9/L. The duration of leukocytosis ranged from 6 to 13 days. Leukapheresis should be considered in the management of symptomatic individuals.

DOSAGE AND ADMINISTRATION

The recommended dosage of Neulasta® is a single subcutaneous injection of 6 mg administered once per chemotherapy cycle. Neulasta® should not be administered in the period between 14 days before and 24 hours after administration of cytotoxic chemotherapy (see PRECAUTIONS).

The 6 mg fixed-dose formulation should not be used in infants, children, and smaller adolescents weighing less than 45 kg.

No dosing adjustment is necessary for renal dysfunction (see CLINICAL PHARMACOLOGY, Special Populations).

Neulasta® should be visually inspected for discoloration and particulate matter before administration. Neulasta® should not be administered if discoloration or particulates are observed.

For method of administration please see patient package insert.

Storage

Neulasta® should be stored refrigerated at 2° to 8°C (36° to 46°F); syringes should be kept in their carton to protect from light until time of use. Shaking should be avoided. Before injection, Neulasta® may be allowed to reach room temperature for a maximum of 48 hours but should be protected from light. Neulasta® left at room temperature for more than 48 hours should be discarded. Freezing should be avoided; however, if accidentally frozen, Neulasta® should be allowed to thaw in the refrigerator before administration. If frozen a second time, Neulasta® should be discarded.

HOW SUPPLIED

Neulasta® is supplied as a preservative-free solution containing 6 mg (0.6 mL) of pegfilgrastim (10 mg/mL) in a single-dose syringe with a 27 gauge, 1/2 inch needle with an UltraSafe® Needle Guard.

Neulasta® is provided in a dispensing pack containing one syringe (NDC 55513-190-01).

Rx Only

This product, its production, and/or its use may be covered by one or more US Patents, including US Patent Nos. 5,824,784; 4,810,643; 4,999,291; 5,582,823; 5,580,755 as well as other patents or patents pending.

REFERENCES

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3. Heil G, Hoelzer D, Sanz MA, et al. A randomized, double-blind, placebo-controlled, phase III study of Filgrastim in remission induction and consolidation therapy for adults with de novo Acute Myeloid Leukemia. Blood. 1997;90:4710-4718.

[RITUXAN®] (RITUXIMAB) Prescribing Information

Boxed warnings

WARNINGS

Fatal Infusion Reactions: Deaths within 24 hours of RITUXAN infusion have been reported. These fatal reactions followed an infusion reaction complex, which included hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, or cardiogenic shock. Approximately 80% of fatal infusion reactions occurred in association with the first infusion. (See WARNINGS and ADVERSE REACTIONS.)

Patients who develop severe infusion reactions should have RITUXAN infusion discontinued and receive medical treatment.

Tumor Lysis Syndrome (TLS): Acute renal failure requiring dialysis with instances of fatal outcome has been reported in the setting of TLS following treatment of non-Hodgkin's lymphoma (NHL) patients with RITUXAN. (See WARNINGS.)

Severe Mucocutaneous Reactions: Severe mucocutaneous reactions, some with fatal outcome, have been reported in association with RITUXAN treatment. (See WARNINGS and ADVERSE REACTIONS.)

DESCRIPTION

The RITUXAN® (Rituximab) antibody is a genetically engineered chimeric murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant B lymphocytes. The antibody is an IgG_1 kappa immunoglobulin containing murine light- and heavy-chain variable region sequences and human constant region sequences. Rituximab is composed of two heavy chains of 451 amino acids and two light chains of 213 amino acids (based on cDNA analysis) and has an approximate molecular weight of 145 kD. Rituximab has a binding affinity for the CD20 antigen of approximately 8.0 nM.

The chimeric anti-CD20 antibody is produced by mammalian cell (Chinese Hamster Ovary) suspension culture in a nutrient medium containing the antibiotic gentamicin. Gentamicin is not detectable in the final product. The anti-CD20 antibody is purified by affinity and ion exchange chromatography. The purification process includes specific viral inactivation and removal procedures. Rituximab drug product is manufactured from bulk drug substance manufactured by Genentech, Inc. (US License No. 1048).

RITUXAN is a sterile, clear, colorless, preservative-free liquid concentrate for intravenous (IV) administration. RITUXAN is supplied at a concentration of 10 mg/mL in either 100 mg (10 mL) or 500 mg (50 mL) single-use vials. The product is formulated for IV administration in 9 mg/mL sodium chloride, 7.35 mg/mL sodium citrate dihydrate, 0.7 mg/mL polysorbate 80, and Water for Injection. The pH is adjusted to 6.5.

CLINICAL PHARMACOLOGY

General

Rituximab binds specifically to the antigen CD20 (human B-lymphocyte-restricted differentiation

antigen, Bp35), a hydrophobic transmembrane protein with a molecular weight of approximately 35 kD located on pre-B and mature B lymphocytes.[1,2] The antigen is also expressed on > 90% of B-cell non-Hodgkin's lymphomas (NHL),[3] but is not found on hematopoietic stem cells, pro-B cells, normal plasma cells or other normal tissues.[4] CD20 regulates an early step(s) in the activation process for cell cycle initiation and differentiation,[4] and possibly functions as a calcium ion channel.[5] CD20 is not shed from the cell surface and does not internalize upon antibody binding.[6] Free CD20 antigen is not found in the circulation.[2]

Preclinical Pharmacology and Toxicology

Mechanism of Action: The Fab domain of Rituximab binds to the CD20 antigen on B lymphocytes, and the Fc domain recruits immune effector functions to mediate B-cell lysis *in vitro*. Possible mechanisms of cell lysis include complement-dependent cytotoxicity (CDC)[7] and antibody-dependent cell mediated cytotoxicity (ADCC). The antibody has been shown to induce apoptosis in the DHL-4 human B-cell lymphoma line.[8]

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

Human Pharmacokinetics/Pharmacodynamics

In patients given single doses at 10, 50, 100, 250 or 500 mg/m^2 as an IV infusion, serum levels and the half-life of Rituximab were proportional to dose.[9] In 14 patients given 375 mg/m^2 as an IV infusion for 4 weekly doses, the mean serum half-life was 76.3 hours (range, 31.5 to 152.6 hours) after the first infusion and 205.8 hours (range, 83.9 to 407.0 hours); after the fourth infusion.[10,11,12] The wide range of half-lives may reflect the variable tumor burden among patients and the changes in CD20-positive (normal and malignant) B-cell populations upon repeated administrations.

RITUXAN at a dose of 375 mg/m^2 was administered as an IV infusion at weekly intervals for 4 doses to 203 patients naive to RITUXAN.[12,13] The mean C_{max} following the fourth infusion was 486 µg/mL (range, 77.5 to 996.6 µg/mL). The peak and trough serum levels of Rituximab were inversely correlated with baseline values for the number of circulating CD20-positive B cells and measures of disease burden. Median steady-state serum levels were higher for responders compared with nonresponders; however, no difference was found in the rate of elimination as measured by serum half-life. Serum levels were higher in patients with International Working Formulation (IWF) subtypes B, C, and D as compared with those with subtype A.[10,13] Rituximab was detectable in the serum of patients 3 to 6 months after completion of treatment.

RITUXAN at a dose of 375 mg/m^2 was administered as an IV infusion at weekly intervals for 8 doses to 37 patients.[14] The mean C_{max} after 8 infusions was 550 µg/mL (range, 171 to 1177 µg/mL). The mean C_{max} increased with each successive infusion through the eighth infusion (Table 1).

Table 1. Rituximab C_{max} Values

Infusion Number	Mean C_{max} µg/mL	Range µg/mL
1	242.6	16.1-581.9
2	357.5	106.8-948.6
3	381.3	110.5-731.2
4	460.0	138.0-835.8
5	475.3	156.0-929.1
6	515.4	152.7-865.2
7	544.6	187.0-936.8
8	550.0	170.6-1177.0

The pharmacokinetic profile of RITUXAN when administered as 6 infusions of 375 mg/m^2 in combination with 6 cycles of CHOP chemotherapy was similar to that seen with RITUXAN alone.[15]

Administration of RITUXAN resulted in a rapid and sustained depletion of circulating and tissue-based B cells. Lymph node biopsies performed 14 days after therapy showed a decrease in the percentage of B cells in seven of eight patients who had received single doses of Rituximab ≥100 mg/m^2. [9] Among the 166 patients in the pivotal study, circulating B cells (measured as CD19-positive cells) were depleted within the first three doses with sustained depletion for up to 6 to 9 months post-treatment in 83% of patients.[13] Of the responding patients assessed (n = 80), 1% failed to show significant depletion of CD19-positive cells after the third infusion of Rituximab as compared to 19% of the nonresponding patients. B-cell recovery began at approximately 6 months following completion of treatment. Median B-cell levels returned to normal by 12 months following completion of treatment.[13]

There were sustained and statistically significant reductions in both IgM and IgG serum levels observed from 5 through 11 months following Rituximab administration. However, only 14% of patients had reductions in IgM and/or IgG serum levels, resulting in values below the normal range.[13]

CLINICAL STUDIES

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

RITUXAN regimens tested include treatment weekly for 4 doses and treatment weekly for 8 doses. Results for studies with a collective enrollment of 296 patients are summarized below (Table 2):

Table 2. Summary of RITUXAN Efficacy Data by Schedule and Clinical Setting (See ADVERSE REACTIONS for Risk Factors Associated with Increased Rates of Adverse Events.)

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

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

[b] Kaplan-Meier projected with observed range.

[c] "+" indicates an ongoing response.

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

Weekly for 4 doses

A multicenter, open-label, single-arm study was conducted in 166 patients with relapsed or refractory low-grade or follicular B-cell NHL who received 375 mg/m^2 of RITUXAN given as an IV infusion weekly for 4 doses.[13] Patients with tumor masses >10 cm or with >5,000 lymphocytes/µL in the peripheral blood were excluded from the study. Results are summarized in Table 2. The median time to onset of response was 50 days and the median duration of response was 11.2 months (range, 1.9 to 42.1+). Disease-related signs and symptoms (including B-symptoms) were present in 23% (39/166) of patients at study entry and resolved in 64% (25/39) of those patients.

In a multivariate analysis, the ORR was higher in patients with IWF B, C, and D histologic subtypes as compared to IWF subtype A (58% vs. 12%), higher in patients whose largest lesion was <5 cm vs. >7 cm (maximum, 21 cm) in greatest diameter (53% vs. 38%), and higher in patients with chemosensitive relapse as compared with chemoresistant (defined as duration of response <3 months) relapse (53% vs. 36%). ORR in patients previously treated with autologous bone marrow transplant was 78% (18/23). The following adverse prognostic factors were *not* associated with a lower response rate: age ≥60 years, extranodal disease, prior anthracycline therapy, and bone marrow involvement.

Weekly for 8 Doses In a multicenter, single-arm study, 37 patients with relapsed or refractory, low-grade NHL received 375 mg/m^2 of RITUXAN weekly for 8 doses. Results are summarized in Table 2. (see ADVERSE REACTIONS, Risk Factors Associated with Increased Rates of Adverse Events.)

Bulky Disease, Weekly for 4 Doses In pooled data from multiple studies of RITUXAN, 39 patients with relapsed or refractory, bulky disease (single lesion >10 cm in diameter), low-grade NHL received 375 mg/m^2 of RITUXAN weekly for 4 doses. Results are summarized in Table 2.[15,16] (For

information on the higher incidence of Grade 3 and 4 adverse events, see ADVERSE REACTIONS, Risk Factors Associated with Increased Rates of Adverse Events.)

Retreatment Weekly for 4 Doses In a multi-center, single-arm study, 60 patients received 375 mg/m^2 of RITUXAN weekly for 4 doses.[17] All patients had relapsed or refractory, low-grade or follicular B-cell NHL and had achieved an objective clinical response to RITUXAN administered 3.8-35.6 months (median 14.5 months) prior to retreatment with RITUXAN. Of these 60 patients, 55 received their second course of RITUXAN, 3 patients received their third course and 2 patients received their second and third courses of RITUXAN in this study. Results are summarized in Table 2.

Diffuse, Large B-Cell, NHL

The safety and effectiveness of RITUXAN were evaluated in three, randomized, active-controlled, open-label, multicenter studies with a collective enrollment of 1854 patients. Patients with previously untreated diffuse, large B-cell, NHL received RITUXAN in combination with cyclophosphamide, doxorubicin, vincristine and prednisone (CHOP) or other anthracycline-based chemotherapy regimens.

Study 1. A total of 632 patients aged ≥60 years with either B-cell NHL Grade F, G, or H by the International Working Formulation classification or DLBCL (including primary mediastinal B-cell lymphoma) in the REAL classification were randomized in a 1:1 ratio to treatment with CHOP or R-CHOP. Patients were given 6 or 8, 21 day cycles of CHOP. Patients in the R-CHOP arm also received 4 or 5 doses of RITUXAN 375 mg/m^2 on Days -7 and -3 (prior to Cycle 1), and 48 to 72 hours pre-Cycle 3, pre-Cycle 5, and pre-Cycle 7 for patients receiving 8 cycles of CHOP induction. The main outcome measure of the study was progression-free survival (PFS), defined as the time from randomization to the first of progression, relapse or death. Responding patients underwent a second randomization to receive RITUXAN or no further therapy.

Among all enrolled patients, 62% had centrally confirmed DLBCL histology, 73% had Stage III-IV disease, 56% had IPI scores ≥2, 86% had ECOG performance status of <2, 57% had elevated LDH levels, and 30% had two or more extranodal disease sites involved. Efficacy results are presented in Table 3. These results reflect a statistical approach which allows for an evaluation of RITUXAN administered in the induction setting that excludes any potential impact of RITUXAN given after the second randomization.

Analysis of results after the second randomization in Study 1 demonstrates that for patients randomized to R-CHOP, additional RITUXAN exposure beyond induction was not associated with further improvements in progression free survival or overall survival.

Study 2. A total of 399 patients with DLBCL, aged ≥60 years, were randomized in a 1:1 ratio to receive CHOP or R-CHOP induction. All patients received up to 8, 3-week cycles of CHOP induction; patients in the R-CHOP arm received RITUXAN 375 mg/m^2 on Day 1 of each cycle. The main outcome measure of the study was event free survival (EFS), defined as the time from randomization to relapse, progression, change in therapy or death from any cause. Among all enrolled patients, 80% had stage III or IV disease, 60% of patients had an age-adjusted IPI ≥2,

80% had ECOG performance status scores <2, 66% had elevated LDH levels, and 52% had extranodal involvement in at least two sites. Efficacy results are presented in Table 3.

Study 3. A total of 823 patients with DLBCL, aged 18-60 years, were randomized in a 1:1 ratio to receive an anthracycline-containing chemotherapy alone or in combination with RITUXAN. The main outcome measure of the study was time to treatment failure (TTF), defined as time from randomization to the earliest of progressive disease, failure to achieve a complete response, relapse or death. Among all enrolled patients, 28% had Stage III-IV disease, 100% had IPI scores of ≤1, 99% had ECOG performance status of <2, 29% had elevated LDH levels, 49% had bulky disease and 34% had extranodal involvement. Efficacy results are presented in Table 3.

Table 3
Efficacy Results in Studies 1, 2, and 3

	Study 1 (n=632)		**Study 2 (n=399)**		**Study 3 (n=823)**	
	CHOP	**R-CHOP**	**CHOP**	**R-CHOP**	**Chemo**	**R-Chemo**
Main outcome	Progression-free survival (years)		Event-free survival (years)		Time to treatment failure (years)	
Median of main outcome measure	1.6	3.1	1.1	2.9	NE[b]	NE[b]
Hazard ratio[d]	0.69[a]		0.60[a]		0.45[a]	
Overall survival at 2 years[c]	63%	74%	58%	69%	86%	95%
Hazard ratio[d]	0.72[a]		0.68[a]		0.40[a]	

[a] Significant at $p<0.05$, 2-sided.

[b] NE=Not reliably estimable.

[c] Kaplan-Meier estimates.

[d] R-CHOP vs. CHOP.

In Study 2, overall survival estimates at 5 years were 58% vs. 46% for R-CHOP and CHOP, respectively.

INDICATIONS AND USAGE

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

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

CONTRAINDICATIONS
RITUXAN is contraindicated in patients with known anaphylaxis or IgE-mediated hypersensitivity to murine proteins or to any component of this product. (See WARNINGS.)

WARNINGS (See BOXED WARNINGS.)

Severe Infusion Reactions (See BOXED WARNINGS, ADVERSE REACTIONS and Hypersensitivity Reactions)

RITUXAN has caused severe infusion reactions. In some cases, these reactions were fatal. These severe reactions typically occurred during the first infusion with time to onset of 30 to 120 minutes. Signs and symptoms of severe infusion reactions may include hypotension, angioedema, hypoxia or bronchospasm, and may require interruption of RITUXAN administration. The most severe manifestations and sequelae include pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, ventricular fibrillation, and cardiogenic shock. In the reported cases, the following factors were more frequently associated with fatal outcomes: female gender, pulmonary infiltrates, and chronic lymphocytic leukemia or mantle cell lymphoma.

Management of severe infusion reactions: The RITUXAN infusion should be interrupted for severe reactions and supportive care measures instituted as medically indicated (e.g., intravenous fluids, vasopressors, oxygen, bronchodilators, diphenhydramine, and acetaminophen). In most cases, the infusion can be resumed at a 50% reduction in rate (e.g., from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Patients requiring close monitoring during first and all subsequent infusions include those with pre-existing cardiac and pulmonary conditions, those with prior clinically significant cardiopulmonary adverse events and those with high numbers of circulating malignant cells ($\geq$ 25,000/mm^3) with or without evidence of high tumor burden.

Tumor Lysis Syndrome [TLS] (See BOXED WARNINGS and ADVERSE REACTIONS) Rapid reduction in tumor volume followed by acute renal failure, hyperkalemia, hypocalcemia, hyperuricemia, or hyperphosphatasemia, have been reported within 12 to 24 hours after the first RITUXAN infusion. Rare instances of fatal outcome have been reported in the setting of TLS following treatment with RITUXAN. The risks of TLS appear to be greater in patients with high numbers of circulating malignant cells ($\geq$ 25,000/mm^3) or high tumor burden. Prophylaxis for TLS should be considered for patients at high risk. Correction of electrolyte abnormalities, monitoring of renal function and fluid balance, and administration of supportive care, including dialysis, should be initiated as indicated. Following complete resolution of the complications of TLS, RITUXAN has been tolerated when re-administered in conjunction with prophylactic therapy for TLS in a limited number of cases.

Hepatitis B Reactivation with Related Fulminant Hepatitis and Other Viral Infections Hepatitis B virus (HBV) reactivation with fulminant hepatitis, hepatic failure, and death has been reported in some patients with hematologic malignancies treated with RITUXAN. The majority of patients received RITUXAN in combination with chemotherapy. The median time to the diagnosis of hepatitis was approximately 4 months after the initiation of RITUXAN and approximately one month after the last dose.

Persons at high risk of HBV infection should be screened before initiation of RITUXAN. Carriers of hepatitis B should be closely monitored for clinical and laboratory signs of active HBV infection and for signs of hepatitis during and for up to several months following RITUXAN therapy. In patients who develop viral hepatitis, RITUXAN and any concomitant chemotherapy should be discontinued and appropriate treatment including antiviral therapy initiated. There are insufficient data regarding the safety of resuming RITUXAN therapy in patients who develop hepatitis subsequent to HBV reactivation.

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

Hypersensitivity Reactions RITUXAN has been associated with hypersensitivity reactions (non-IgE-mediated reactions) which may respond to adjustments in the infusion rate and in medical management. Hypotension, bronchospasm, and angioedema have occurred in association with RITUXAN infusion (see Severe Infusion Reactions). RITUXAN infusion should be interrupted for severe hypersensitivity reactions and can be resumed at a 50% reduction in rate (e.g., from 100 mg/hr to 50 mg/hr) when symptoms have completely resolved. Treatment of these symptoms with diphenhydramine and acetaminophen is recommended; additional treatment with bronchodilators or IV saline may be indicated. In most cases, patients who have experienced non-life-threatening hypersensitivity reactions have been able to complete the full course of therapy. (See DOSAGE and ADMINISTRATION.) Medications for the treatment of hypersensitivity reactions, e.g., epinephrine, antihistamines and corticosteroids, should be available for immediate use in the event of a reaction during administration.

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

Renal (See BOXED WARNINGS: Tumor Lysis Syndrome [TLS] and ADVERSE REACTIONS) RITUXAN administration has been associated with severe renal toxicity including acute renal failure requiring dialysis and in some cases, has led to a fatal outcome. Renal toxicity has occurred in patients with high numbers of circulating malignant cells (>25,000/mm^3) or high tumor burden who experience tumor lysis syndrome (see Tumor Lysis Syndrome) and in patients administered concomitant cisplatin therapy during clinical trials. The combination of cisplatin and RITUXAN is not an approved treatment regimen. If this combination is used in clinical trials *extreme caution* should be exercised; patients should be monitored closely for signs of renal failure. Discontinuation of RITUXAN should be considered for those with rising serum creatinine or oliguria.

Severe Mucocutaneous Reactions (See BOXED WARNINGS) Mucocutaneous reactions, some with fatal outcome, have been reported in patients treated with RITUXAN. These reports include paraneoplastic pemphigus (an uncommon disorder which is a manifestation of the patient's underlying malignancy),[18] Stevens-Johnson syndrome, lichenoid dermatitis, vesiculobullous dermatitis, and toxic epidermal necrolysis. The onset of the reaction in the reported cases has varied from 1 to 13 weeks following RITUXAN exposure. Patients experiencing a severe mucocutaneous reaction should not receive any further infusions and seek prompt medical evaluation. Skin biopsy may help to distinguish among different mucocutaneous reactions and guide subsequent treatment. The safety of readministration of RITUXAN to patients with any of these mucocutaneous reactions has not been determined.

Bowel Obstruction and Perforation Abdominal pain, bowel obstruction and perforation, in some cases leading to death, were observed in patients receiving RITUXAN in combination with chemotherapy for DLBCL. In post-marketing reports, which include both patients with low-grade or follicular NHL and DLBCL, the mean time to onset of symptoms was 6 days (range 1–77) in patients with documented gastro-intestinal perforation. Complaints of abdominal pain, especially early in the course of treatment, should prompt a thorough diagnostic evaluation and appropriate treatment.

PRECAUTIONS
Laboratory Monitoring

Because RITUXAN targets all CD20-positive B lymphocytes, malignant and nonmalignant, complete blood counts (CBC) and platelet counts should be obtained at regular intervals during RITUXAN therapy and more frequently in patients who develop cytopenias (see ADVERSE REACTIONS). The duration of cytopenias caused by RITUXAN can extend well beyond the treatment period.

Drug/Laboratory Interactions

There have been no formal drug interaction studies performed with RITUXAN. However, renal toxicity was seen with this drug in combination with cisplatin in clinical trials. (See WARNINGS, Renal.)

Immunization
The safety of immunization with live viral vaccines following RITUXAN therapy has not been studied. The ability to generate a primary or anamnestic humoral response to vaccination is currently being studied.

Carcinogenesis, Mutagenesis, Impairment of Fertility

No long-term animal studies have been performed to establish the carcinogenic or mutagenic potential of RITUXAN, or to determine its effects on fertility in males or females. Individuals of childbearing potential should use effective contraceptive methods during treatment and for up to 12 months following RITUXAN therapy.

Pregnancy Category C

Animal reproduction studies have not been conducted with RITUXAN. It is not known whether RITUXAN can cause fetal harm when administered to a pregnant woman or whether it can affect reproductive capacity. Human IgG is known to pass the placental barrier, and thus may potentially cause fetal B-cell depletion; therefore, RITUXAN should be given to a pregnant woman only if clearly needed.

Nursing Mothers

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

Pediatric Use

The safety and effectiveness of RITUXAN in pediatric patients have not been established.

Geriatric Use

Among patients with DLBCL in three randomized, active-controlled trials, 927 patients received RITUXAN in combination with chemotherapy. Of these, 396 (43%) were age 65 or greater and 123 (13%) were age 75 or greater. No overall differences in effectiveness were observed between these subjects and younger subjects. However, elderly patients were more likely to experience cardiac adverse events, mostly supraventricular arrhythmias. Serious pulmonary adverse events were also more common among the elderly, including pneumonia and pneumonitis.

Among the 331 patients with low-grade or follicular lymphoma enrolled in clinical studies of single agent RITUXAN, 24% were 65 to 75 years old and 5% were 75 years old and older. No overall differences in safety or effectiveness were observed between these subjects and younger subjects.

ADVERSE REACTIONS

Because clinical trials are conducted under widely varying conditions, adverse reaction rates observed in the clinical trials of a drug cannot be directly compared to rates in the clinical trials of another drug and may not reflect the rates observed in practice. The adverse reaction information from clinical trials does, however, provide a basis for identifying the adverse events that appear to be related to drug use and for approximating rates.

The overall safety database for RITUXAN is based on clinical trial data from 1283 patients with NHL, who received RITUXAN either as a single agent or in combination with chemotherapy. Additional safety information was obtained from post-marketing safety surveillance. The most common adverse reactions were infusion reactions (see INFUSION REACTIONS below).

The following serious adverse reactions, some with fatal outcomes, have been reported in patients treated with RITUXAN (see BOXED WARNINGS and WARNINGS): severe or fatal infusion reactions, tumor lysis syndrome, severe mucocutaneous reactions, hepatitis B reactivation with fulminant hepatitis, other viral infections, hypersensitivity reactions, cardiac arrhythmias, renal toxicity, bowel obstruction and perforation.

Except as noted, adverse events described below occurred in the setting of relapsed or refractory, low-grade or follicular, CD20-positive, B-cell, NHL and are based on 356 patients treated in nonrandomized, single-arm studies of RITUXAN administered as a single agent. Most patients received RITUXAN 375 mg/m^2 weekly for 4 doses.

Infusion Reactions (See BOXED WARNINGS and WARNINGS)

Mild to moderate infusion reactions consisting of fever and chills/rigors occurred in the majority of patients during the first RITUXAN infusion. Other frequent infusion reaction symptoms included nausea, pruritus, angioedema, asthenia, hypotension, headache, bronchospasm, throat irritation, rhinitis, urticaria, rash, vomiting, myalgia, dizziness, and hypertension. These reactions generally occurred within 30 to 120 minutes of beginning the first infusion, and resolved with slowing or interruption of the RITUXAN infusion and with supportive care (diphenhydramine, acetaminophen, IV saline, and vasopressors). The incidence of infusion reactions was highest during the first infusion (77%) and decreased with each subsequent infusion (30% with fourth infusion and 14% with eighth infusion). Injection site pain was reported in less than 5% of patients.

Infectious Events (See WARNINGS: Hepatitis B Reactivation with Related Fulminant Hepatitis and Other Viral Infections)

RITUXAN induced B-cell depletion in 70% to 80% of patients and was associated with decreased serum immunoglobulins in a minority of patients; the lymphopenia lasted a median of 14 days (range, 1 to 588 days). Infectious events occurred in 31% of patients: 19% of patients had bacterial infections, 10% had viral infections, 1% had fungal infections, and 6% were unknown infections. Incidence is not additive because a single patient may have had more than one type of infection. Serious infectious events (Grade 3 or 4), including sepsis, occurred in 2% of patients.

Hematologic Events

Grade 3 and 4 cytopenias were reported in 48% of patients treated with RITUXAN; these include: lymphopenia (40%), neutropenia (6%), leukopenia (4%), anemia (3%), and thrombocytopenia (2%). The median duration of lymphopenia was 14 days (range, 1 to 588 days) and of neutropenia was 13 days (range, 2 to 116 days). A single occurrence of transient aplastic anemia (pure red cell aplasia) and two occurrences of hemolytic anemia following RITUXAN therapy were reported.

Pulmonary Events

135 patients (38%) experienced pulmonary events in clinical trials. The most common respiratory system adverse events experienced were increased cough, rhinitis, bronchospasm, dyspnea, and sinusitis. In both clinical studies and post-marketing surveillance, there have been a limited number of reports of bronchiolitis obliterans presenting up to 6 months post-RITUXAN infusion and a limited number of reports of pneumonitis (including interstitial pneumonitis) presenting up to 3 months post-RITUXAN infusion, some of which resulted in fatal outcomes. The safety of resumption or continued administration of RITUXAN in patients with pneumonitis or bronchiolitis obliterans is unknown.

Immunogenicity
The observed incidence of antibody positivity in an assay is highly dependent on the sensitivity and specificity of the assay and may be influenced by several factors including sample handling, concomitant medications, and underlying disease. For these reasons, comparison of the incidence of antibodies to RITUXAN with the incidence of antibodies to other products may be misleading.

In clinical studies of patients with low-grade or follicular NHL receiving single-agent RITUXAN, human antichimeric antibody (HACA) was detected in 4 of 356 (1.1%) patients and 3 had an objective clinical response. These data reflect the percentage of patients whose test results were considered positive for antibodies to RITUXAN using an enzyme-linked immunosorbant assay (limit of detection = 7 ng/mL).

Single Agent RITUXAN for Relapsed or Refractory, Low-Grade or Follicular, CD20-Positive, B-Cell, NHL

Study subjects ranged from 22 to 81 years of age. Sixty percent were male; 93% were Caucasian, 1% were African American, 2% were Hispanic, 2% were Asian, and 2% were from other racial groups. Table 4 lists the most common, as well as Grade 3 and 4, adverse events observed.

Table 4
Incidence of Adverse Events in ≥ 5% of Patients with Relapsed or Refractory, Low-Grade or Follicular NHL, Receiving Single-agent RITUXAN (N=356)[a,b]

	All Grades (%)	Grade 3 and 4 (%)
Any Adverse Events	**99**	**57**
Body as a Whole	**86**	**10**
Fever	53	1
Chills	33	3
Infection	31	4
Asthenia	26	1

Headache	19	1
Abdominal Pain	14	1
Pain	12	1
Back Pain	10	1
Throat Irritation	9	0
Flushing	5	0
Cardiovascular System	**25**	**3**
Hypotension	10	1
Hypertension	6	1
Digestive System	**37**	**2**
Nausea	23	1
Diarrhea	10	1
Vomiting	10	1
Hemic and Lymphatic System	**67**	**48**
Lymphopenia	48	40
Leukopenia	14	4
Neutropenia	14	6
Thrombocytopenia	12	2
Anemia	8	3
Metabolic and Nutritional Disorders	**38**	**3**
Angioedema	11	1
Hyperglycemia	9	1
Peripheral Edema	8	0
LDH Increase	7	0
Musculoskeletal System	**26**	**3**
Myalgia	10	1
Arthralgia	10	1
Nervous System	**32**	**1**
Dizziness	10	1
Anxiety	5	1
Respiratory System	**38**	**4**

Increased Cough	13	1
Rhinitis	12	1
Bronchospasm	8	1
Dyspnea	7	1
Sinusitis	6	0
Skin and Appendages	**44**	**2**
Night Sweats	15	1
Rash	15	1
Pruritus	14	1
Urticaria	8	1

[a] Adverse Events observed up to 12 months following RITUXAN.

[b] Adverse Events graded for severity by NCI-CTC criteria.[19]

Risk Factors Associated with Increased Rates of Adverse Events

Administration of RITUXAN weekly for 8 doses resulted in higher rates of Grade 3 and 4 adverse events[14] overall (70%) compared with administration weekly for 4 doses (57%). The incidence of Grade 3 or 4 adverse events was similar in patients retreated with RITUXAN compared with initial treatment (58% and 57%, respectively). The incidence of the following clinically significant adverse events was higher in patients with bulky disease (lesions ≥ 10 cm) (N = 39) versus patients with lesions < 10 cm (N = 195): abdominal pain, anemia, dyspnea, hypotension, and neutropenia.

RITUXAN in Combination with Chemotherapy for DLBCL

Except as noted, adverse events described in the setting of DLBCL are based on three randomized, active-controlled clinical trials in which 927 patients received RITUXAN in combination with chemotherapy and 802 received chemotherapy alone. Detailed safety data collection was primarily limited to Grade 3 and 4 adverse events and serious adverse events.

The population varied from 18 to 92 years of age and 55% were male; racial distribution was collected only for Study 1 (see CLINICAL STUDIES section) where 90% of patients were Caucasian, 5% were African American, 3% were Hispanic and 2% were from other racial groups. Patients received 4–8 doses of RITUXAN at 375 mg/m^2.

The following adverse events, regardless of severity, were reported more frequently (≥5%) in patients age ≥60 years receiving R-CHOP as compared to CHOP alone: cardiac disorder (29% vs. 21%), pyrexia (56% vs. 46%), chills (13% vs. 4%) and lung disorder (31% vs. 24%). In one of these studies (Study 2), more detailed assessment of cardiac toxicity revealed that supraventricular arrhythmias or tachycardia accounted for most of the difference in cardiac disorders, with 4.5% vs. 1.0% incidences for R-CHOP and CHOP, respectively.

The following Grade 3 or 4 adverse events were reported more frequently among patients in the R-CHOP arm compared with those in the CHOP arm: thrombocytopenia (9% vs. 7%) and lung disorder (6% vs. 3%). Other severe adverse events reported more commonly among patients receiving R-CHOP in one or more studies were viral infection, neutropenia and anemia.

Post-Marketing Reports

The following adverse reactions have been identified during post-approval use of RITUXAN. Because these reactions are reported voluntarily from a population of uncertain size, it is not always possible to reliably estimate their frequency or establish a causal relationship to drug exposure. Decisions to include these reactions in labeling are typically based on one or more of the following factors: (1) seriousness of the reaction, (2) frequency of reporting, or (3) strength of causal connection to RITUXAN.

Hematologic: prolonged pancytopenia, marrow hypoplasia, and late onset neutropenia, hyperviscosity syndrome in Waldenstrom's macroglobulinemia.

Cardiac: fatal cardiac failure.

Immune/Autoimmune Events: uveitis, optic neuritis, systemic vasculitis, pleuritis, lupus-like syndrome, serum sickness, polyarticular arthritis and vasculitis with rash.

Infection: increased in fatal infections in HIV-associated lymphoma.

Skin: severe mucocutaneous reactions.

Gastrointestinal: bowel obstruction and perforation.

OVERDOSAGE
There has been no experience with overdosage in human clinical trials. Single doses of up to 500 mg/m^2 have been given in dose-escalating clinical trials.[9]

DOSAGE AND ADMINISTRATION

Relapsed or Refractory, Low-Grade or Follicular, CD20-Positive, B-Cell, Non-Hodgkin's Lymphoma The recommended dose of RITUXAN is 375 mg/m^2 IV infusion once weekly for 4 or 8 doses.

Retreatment Therapy. The recommended dose of RITUXAN is 375 mg/m^2 IV infusion once weekly for 4 doses in responding patients who develop progressive disease after previous RITUXAN therapy. Currently there are limited data concerning more than 2 courses.

Diffuse Large B-Cell NHL

The recommended dose of RITUXAN is 375 mg/m^2 IV per infusion given on Day 1 of each cycle of chemotherapy for up to 8 infusions.

RITUXAN as a Component of Zevalin™ (Ibritumomab Tiuxetan) Therapeutic Regimen
As a required component of the Zevalin therapeutic regimen, RITUXAN 250 mg/m^2 should be infused within 4 hours prior to the administration of Indium-111- (In-111-) Zevalin and within 4 hours prior to the administration of Yttrium90- (Y-90-) Zevalin. Administration of RITUXAN and In-111-Zevalin should precede RITUXAN and Y-90-Zevalin by 7–9 days. Refer to the Zevalin package insert for full prescribing information regarding the Zevalin therapeutic regimen. RITUXAN may be administered in an outpatient setting. **DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS. (See Administration.)**

Instructions for Administration

Preparation for Administration. Use appropriate aseptic technique. Withdraw the necessary amount of RITUXAN and dilute to a final concentration of 1 to 4 mg/mL into an infusion bag containing either 0.9% Sodium Chloride, USP, or 5% Dextrose in Water, USP. Gently invert the bag to mix the solution. Discard any unused portion left in the vial. Parenteral drug products should be inspected visually for particulate matter and discoloration prior to administration.

RITUXAN solutions for infusion may be stored at 2-8°C (36-46°F) for 24 hours. RITUXAN solutions for infusion have been shown to be stable for an additional 24 hours at room temperature. However, since RITUXAN solutions do not contain a preservative, diluted solutions should be stored refrigerated (2-8°C). No incompatibilities between RITUXAN and polyvinylchloride or polyethylene bags have been observed.

Administration: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS
Infusion and hypersensitivity reactions may occur (see BOXED WARNINGS, WARNINGS, and ADVERSE REACTIONS). Premedication consisting of acetaminophen and diphenhydramine should be considered before each infusion of RITUXAN. Premedication may attenuate infusion reactions. Since transient hypotension may occur during RITUXAN infusion, consideration should be given to withholding antihypertensive medications 12 hours prior to RITUXAN infusion.

First Infusion. The RITUXAN solution for infusion should be administered intravenously at an initial rate of 50 mg/hr. RITUXAN should not be mixed or diluted with other drugs. If hypersensitivity or infusion reactions do not occur, escalate the infusion rate in 50 mg/hr increments every 30 minutes, to a maximum of 400 mg/hr. If a hypersensitivity (non-IgE-mediated) or an infusion reaction develops, the infusion should be temporarily slowed or interrupted (see BOXED WARNINGS and WARNINGS). The infusion can continue at one-half the previous rate upon improvement of patient symptoms.

Subsequent Infusions. If the patient tolerated the first infusion well, subsequent RITUXAN infusions can be administered at an initial rate of 100 mg/hr, and increased by 100 mg/hr increments at 30-minute intervals, to a maximum of 400 mg/hr as tolerated. If the patient did not tolerate the first infusion well, follow the guidelines under First Infusion.

Stability and Storage

RITUXAN vials are stable at 2-8°C (36-46°F). Do not use beyond expiration date stamped on carton. RITUXAN vials should be protected from direct sunlight. Do not freeze or shake. Refer to the "Preparation for Administration" section for information on the stability and storage of solutions of RITUXAN diluted for infusion.

HOW SUPPLIED

RITUXAN® (Rituximab) is supplied as 100 mg and 500 mg of sterile, preservative-free, single-use vials.

Single unit 100 mg carton: Contains one 10 mL vial of RITUXAN (10 mg/mL). NDC 50242-051-21

Single unit 500 mg carton: Contains one 50 mL vial of RITUXAN (10 mg/mL). NDC 50242-053-06

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APPENDIX B

WHO Classification of Hematopoietic and Lymphoid Tumors: *B-cell Neoplasms*

Indolent	Aggressive	Very Aggressive
• Chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma	• Prolymphocytic leukemia	• Precursor B-lymphoblastic lymphoma/leukemia
• Lymphoplasmacytic/ Waldenström's macroglobulinemia (WM)	• Plasmacytoma/ multiple myeloma	• Burkitt lymphoma/ B-cell acute leukemia
• Hairy cell leukemia	• Mantle cell	• Plasma cell leukemia

• Marginal zone lymphoma
  ◦ Extranodal mucosa-associated lymphoid tissue (MALT)
  ◦ Nodal
  ◦ Splenic
	• Follicle center lymphoma, follicular, grade III	

• Follicle center lymphoma, follicular, grade I-II	• Diffuse large B-cell lymphoma (DLBCL)	
	• Primary mediastinal large B-cell lymphoma	

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APPENDIX C

Criteria for Evaluation of Response

1. Response Classifications

After study entry, disease activity evaluations will be made and recorded by using the following criteria and designated codes:

A. Complete Response (CR): During observation no disease is apparent, including measurable and non-measurable disease, and no evidence of disease is observed for at least 28 days, as confirmed by a second assessment following the original observation of no disease. All nodes visualized on imaging studies or palpable on exams must have regressed to normal size (≤1.5 cm in their greatest transverse diameter for nodes > 1.5 cm before therapy). Previously involved nodes that were 1.1 to 1.5 cm in their greatest transverse diameter before treatment must have decreased to ≤ 1 cm in their greatest transverse diameter after treatment, or by more than 75% in the sum of the products of the greatest diameters (SPD). (A^1) = The patient must also be free from symptoms related to lymphoma, if present before therapy with no worsening in performance status from baseline. (A^2) = Bone marrow, if initially positive at baseline, must be histologically negative for lymphoma and the liver and spleen, if enlarged due to lymphoma at baseline, should be normalized.

In addition, gallium-avid-disease or PET-positive at baseline that converts to negative will be accepted as confirmation of response, regardless of nodal size.

B. Partial Response (PR): A 50% or greater decrease from baseline in the sum of the products of the longest perpendicular diameters of all the measured lesions is noted for at least 28 days as confirmed by a second assessment following the observation of the > or = to 50% decrease. Additionally, no appearance of new lesions is noted.

C. CR/Unconfirmed (CRu): Includes those patients who fulfill criteria (A^1) and (A^2) see above, but with one or more of the following features:

1. A residual lymph node mass greater than 1.5 cm in greatest transverse diameter then that has regressed by more than 75% in the SPD. Individual nodes that were previously confluent must have regressed by more than 75% in their SPD compared with the size of the original mass.

2. Indeterminate bone marrow (increased number or size of aggregates without cytologic or architectural atypia).

D. Stable Disease (SD): Patients have stable disease if they neither exhibit at least a 50% decrease in the sum of the products of the longest perpendicular diameters of all the measured lesions, nor exhibit at least a 50% increase in the sum of the products of the longest perpendicular diameters of all the measured lesions. In addition no new lesions may have appeared.

E. Progressive Disease (PD): Progressive disease will be assessed with reference to baseline values for measurable lesions unless a decrease in size has been observed, in which case progression will be measured from the nadir of the sum of the products of the longest perpendicular diameters of all measured lesions.

Any single observation of a >25% increase in the sum of the sizes of all sentinel lesions, a 50% or greater increase in the size of any single lesion or the appearance of a new lesion constitutes progressive disease. A statement by the investigator indicating that clinical progressive disease has occurred may be used to signify progressive disease, as well as any death for which disease has occurred must include the date of disease progression and confirmatory documentation, i.e. of non-sentinel lesions or non-measurable disease, etc.

RESPONSE CRITERIA FOR NON-HODGKIN'S LYMPHOMA

Response Category	Physical Examination	Lymph Nodes	Lymph Node Masses	Bone Marrow
CR	Normal	Normal	Normal	Normal
CRu	Normal	Normal	Normal	Indeterminate
CRu	Normal	Normal	> 75% decrease	Normal
PR	Normal	Normal	Normal	Positive
PR	Normal	≥ 50% decrease	≥ 50% decrease	Irrelevant
PR	Decrease in liver/spleen	≥ 50% decrease	≥ 50% decrease	Irrelevant
Relapsed/ Progression	Enlarging liver/spleen; new sites	New or increased	New or increased	Reappearance

2. Measures of Duration

A. Response Interval: measured from the date when the response is first noted until the last date at which the measurements satisfy the requirements for a partial or complete response. An ongoing response is defined as a responder for whom no end of response can be determined. In this case, the duration of response is considered as the last date when there is evidence of a continuing response.
B. Progressive Disease (PD)-Free Interval: measured from the date when a CR or PR is first noted to the first date at which progressive disease is observed. An ongoing PD-Free Interval occurs when there is a responder for whom progressive disease has not yet been noted.
C. Time to Progression: measured from the date of first study treatment to the first date when progressive disease is documented.
D. Survival: duration of survival will be dated from the first day of treatment.

3. Sentinel Lesions

A minimum of one and maximum of six sentinel lesions will be selected for determining the efficacy of study treatment. All sentinel lesions must be measured at baseline by the method, which will be used, for routine tumor measurements during the study. Sentinel lesions are defined as those lesions existing at baseline that most clearly represent the state of disease in the clinical judgment of the investigator, and need to be measurable bi dimensionally. Sentinel lesions need to be at least 1.0 cm x 1.0 cm in size selected before treatment begins, and should not be in previously irradiated fields (unless progression has occurred). The liver (span) itself cannot be used as a sentinel lesion, although lesions within the liver are acceptable. The minimum acceptable size of the sentinel lesions will depend on the number and dimension as outlined in the following table:

Number of Lesions	Dimensions	Minimum Size
≥1	Bi-dimensional	1.0 x 1.0 cm

4. Definition of Measurable Disease

The extent of disease is measurable if the patient has one or more lesions that are clearly demarcated and may be represented with clearly defined margins as measured in centimeters. These measurements will be bi-dimensional (two perpendicular measures per lesion). Skin lesions must be documented by medical photography.

5. Measurement of Sentinel Lesions:

All sentinel lesions must be measured at baseline by the method that will be used for routine tumor measurements during the study. Each sentinel lesion will be consistently measured bi-dimensionally for the duration of the study. In addition, the routine method of measurement for any sentinel lesions, e.g., X-ray, PET scan, CT scan, etc., should remain the same for each evaluation throughout the study. All measurements should be recorded in metric units, using a ruler or calipers.

A. Bi-dimensional Disease: measured by two perpendicular measurements of the lesion. The first measurement is the longest span of the lesion, and the other measurement is the longest measurement perpendicular to the first measurement. The size of the lesion is the product of these two measurements.

B. Overall Size of Sentinel Lesions: the sum of the products of the two dimensions of each bi dimensional lesion(s).

C. Uni dimensional sites of disease may be used only in addition to bi dimensionally measured disease >1.0 x 1.0 cm. Record the single dimension as measured by ruler or caliper.

APPENDIX D

Performance Status Scale

Status[a,b] (Karnofsky)	Scale		"WHO" Status[c,d] (Zubrod-ECOG)
Normal, no complaints	100	0	Normal activity
Able to carry on normal activity; minor signs or symptoms of disease	90	1	Symptoms, but ambulatory
Normal activities for effort	80		
Cares for self, unable to carry on normal activity or to do active work	70	2	Some bed time, but in bed <50% of normal daytime
Requires occasional assistance, but able to care for most of own needs	60		
Requires considerable assistance and frequent medical care	50	3	Needs to be in bed .50% of normal daytime
Disabled, requires special care and assistance	40		
Severely disabled, hospitalization indicated though death not imminent	30	4	Unable to get out of bed
Very sick, hospitalization necessary, active supportive treatment necessary	20		
Moribund	10		
Dead	0		

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APPENDIX E Study Flow Chart

Table 2. Calendar of procedures before and during Pegfilgrastim in combination with rituximab

Week	-4 to 0	1	3	5	7	9	11	13	15	17	19	21	23	25	27	29	31	33	35	37	39	43
Medical history	◙	◙	◙	◙	◙				◙				◙				◙				◙	◙
Physical exam	◙	◙	◙	◙	◙				◙				◙				◙				◙	◙
Height and body weight (BSA calculation) at screening only; thereafter body weight only	◙	◙	◙	◙	◙				◙				◙				◙				◙	◙
ECOG performance status	◙	◙	◙	◙	◙				◙				◙				◙				◙	◙
CBC.	◙	◙	◙	◙	◙				◙				◙				◙				◙	◙
Chemistry panel	◙	◙	◙	◙	◙				◙				◙				◙				◙	◙
Pregnancy test (if applicable)	◙																					
Bone marrow and peripheral blood studies [a]	◙																					◙
Lymph node biopsy [b]	◙																					
Beta 2 microglobulin (β2M)	◙						◙								◙							◙
Urinalysis	◙																					
Tumor measurements [c]	◙						◙								◙							◙
Imaging studies [c, d] (PET scan, CT scan[g] or MRI)	◙																					◙
Quantitative immunoglobulins (IgG, IgA, IgM)	◙						◙								◙							◙
Screening of patients for HIV, HBV and HCV	◙																					
Correlative studies [e]	◙	◙	◙	◙	◙				◙				◙				◙				◙	
Pegfilgrastim [f]		◙	◙	◙	◙				◙				◙				◙				◙	
Rituximab		◙	◙	◙	◙				◙				◙				◙				◙	

[a] Repeat bone marrow biopsy at the end of therapy only if was involved with lymphoma during the staging work up or clinically indicated by treating physician.

[b] Lymph node biopsy and sample for surface antigen markers (e.g. CD20, CD19, etc.); PCR for Ig gene rearrangement in FL patients; and Bcl-2 detection for patients with accessible and enlarged lymph node. As long as they do not have any medical contraindications for biopsy as determined by the treating physician. For patients with no accessible tumor lesions or with medical contraindications for surgical procedure, archived pathological material will be analyzed for confirmation of diagnosis, CD20 expression and in cases of FL for IgH gene rearrangement and Bcl-2 studies

[c] Tumor measurements and Imaging studies – If patient has a response (PR or CR), the sentinel lesions chosen at baseline will be reassessed 1 to 4 months later to confirm the response. [d] Imaging studies will be done on weeks 11, 27 and 43. [e] Various laboratory parameters will be analyzed for each patient enrolled in the study before treatment (baseline) and before each dose of rituximab [f] Pegfilgrastim will be administered sq 3 days (day 1 of each treatment week) before each dose of rituximab (day 4 of each treatment week) g CT scans will be performed during the first two years of follow-up only.

APPENDIX E (contd.) **Study Flow Chart**

**Table 3. Calendar of Procedures During 4-year (48 month) Follow-Up –
For Assessment of Time to Progression and Survival Only, for 4 years (48 months) after the last study treatment**

	Year 1 of Follow-Up	Year 2 of Follow-Up	Year 3 of Follow-Up	Year 4 of Follow-Up
Medical history	Every 4 months	Every 6 months	Every 6 months	
Physical exam	Every 4 months	Every 6 months	Every 6 months	
ECOG performance status	Every 4 months	Every 6 months	Every 6 months	
Imaging studies (PET scan if clinically indicated, CT scan or MRI)	Every 4 months	Every 6 months	Every 6 months No CT scans in year 3	
Hematology: CBC with differential and platelet count.	Every 4 months	Every 6 months	Every 6 months	
Serum Chemistries: creatinine, uric acid, total bilirubin, alkaline phosphatase, LDH, total protein, albumin, glucose, AST (SGOTY), calcium, phosphate, sodium, potassium, and BUN	Every 4 months	Every 6 months	Every 6 months	
Laboratory studies: Peripheral blood (and bone marrow samples when available) for bcl-2 testing if the baseline assessment was positive for this marker.	Once a year	Once a year	Once a year	Once a year