TITLE: Phase I/Ib Study of Carfilzomib Plus Rituximab Plus Ifosfamide Plus Carboplatin Plus Etoposide (C-R-ICE) in Patients with Relapsed/Refractory Diffuse Large B-Cell Lymphoma (DLBCL)

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Sponsor:	**Roswell Park**
Industry Supporter:	**Amgen, Inc. (20159860)**

NCT#01959698

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SYNOPSIS

Title / Phase	Phase I/Ib Study of Carfilzomib Plus Rituximab Plus Ifosfamide Plus Carboplatin Plus Etoposide (C-R-ICE) in Patients with Relapsed/Refractory Diffuse Large B-Cell Lymphoma (DLBCL)
Roswell Park Study Number	I 240813
Roswell Park Investigator	Francisco J. Hernandez-Ilizaliturri, MD
Sponsor	Amgen, Inc. (20159860)
Study Drug	Carfilzomib
Objectives	**Phase I Primary Objectives** • To estimate the maximum tolerated dose (MTD) and examine the dose-limiting toxicities of carfilzomib when administered in combination with rituximab, ifosfamide, carboplatin, and etoposide (C-R-ICE) in patients with relapsed/refractory DLBCL. • To assess the toxicity of dose regimen using the CTEP NCI Common Terminology Criteria for Adverse Events (CTCAE Version 4.0). **Phase Ib Primary Objectives (Expansion Cohort)** • To evaluate the safety of carfilzomib (given at maximum tolerated dose [MTD] as determined in Phase I component of this study) in combination with R-ICE salvage therapy in relapsed/refractory DLBCL patients. • To achieve an overall response rate (CR and PR) of 70% after 3 cycles of C-R-ICE in patients between the ages of 18 to 75 with relapsed/refractory CD20-positive DLBCL previously treated with rituximab-based immunochemotherapy (e.g., R-CHOP, R-EPOCH, R-HyperCVAD, etc.) induction. **Secondary Objectives** • To determine the feasibility of successful mobilization of autologous stem cells (i.e., minimum of 2 x 10^6 CD34+ cells/kg should be collected) to be used for ASCT. (if applicable) • To determine toxicities associated with C-R-ICE salvage therapy. • To determine the TTP, PFS, and OS followed by ASCT (if applicable); Disease-free survival in CR patients. • To determine the pharmacokinetics/pharmacodynamics relationship between carfilzomib's degree of proteasome inhibition and response rate along with the time course of thrombocytopenia. • To study differences in clinical outcomes between GCB and non-GCB relapsed/refractory DLBCL following therapy with carfilzomib and R-ICE. • Correlative translational research studies to include: phenotypic/genotypic analysis and functional activity (i.e., ADCC and CMC) of patient's peripheral blood mononuclear "effector" cells (PBMC), as well as ex vivo analysis of sensitivity of primary tumor cells to various combinations of carfilzomib versus bortezomib +/- rituximab;, enzymatic assay for chymotrypsin-like activity to determine the degree of proteasome inhibition in primary DLBCL patient samples and patient PBMC specimens;

	explorative analysis to identify potential factors predictive of response to therapy will be performed (as part of an NIH-supported R01 grant: R01 CA136907-0Ab; PI: Francisco J. Hernandez-Ilizaliturri, MD).
Study Design	This is multi-center open-label, prospective Phase I/Ib clinical trial evaluating the safety, efficacy, and pharmacokinetics/pharmacodynamics of Carfilzomib + standard R-ICE combination therapy in adult patients with relapsed/refractory diffuse large B-cell lymphoma. The statistical design is based on a dose-finding algorithm to determine the maximal tolerated dose (MTD) of the study regimen and an assessment of the efficacy of the treatment for patients as compared to an external standard, i.e., R-ICE historical control. All enrolled patients will receive the study drug and initiation of the treatment should take place as soon as possible following study enrollment. A total of up to 30 evaluable patients will be enrolled in the Phase I/Ib component
Target Accrual and Study Duration	A maximum of 21 patients for Phase I and 9 patients for Phase Ib expansion cohort will be enrolled at Roswell Park. The number of subjects required is a function of a currently unknown dose-toxicity relationship. Accrual is expected to take up to 10 years.
Study Procedures	Disease Evaluation: Prior to study, at time of restaging; between Cycle 2 Day 15 to Cycle 2 Day 28 following Cycle 3 but before ASCT and at the end of treatment evaluation, then every 4 months (±2 weeks) for 1 year, then every 6 months (±2 weeks) for 2 years, then once a year (±2 weeks) for 2 years for a total of 5 years, then as clinically indicated. The follow up period will begin at the end of study evaluation visit for patients that became ineligible for transplant for any reason but did not meet criteria for disease progression. The follow up period for patients that go on to transplant will begin at the Day +100 bone marrow biopsy. Patients that progress at any time will be followed for survival status only on a yearly basis. Adverse Events: From first dose of study drug until 30 days after receiving last dose of study drug. Hematology and Chemistry: Prior to study, Day 1 of each cycle, and post treatment follow-up. Performance Status: Prior to study, Day 1 of each cycle, and post treatment follow-up. History and Physical Examination Prior to study, Day 1 of each cycle, and post treatment follow-up. Electrocardiogram: Prior to study
Statistical Analysis	• Phase I: The actual sample size for the Phase I population depends on the unknown dose-toxicity profile of the treatment and the maximum sample size is 21 patients. • Phase Ib (Dose expansion cohort): The sample size calculation for the Phase Ib was based on testing the hypotheses concerning the proportion of the population who experience an objective response (i.e., CR or PR) described above. This design requires a total of up to 30 patients (it will include the patients treated in the Phase I phase and 9 patients enrolled in the dose expansion cohort). This will achieve approximately 80.1% power to detect differences of 20 percentage points (50% versus 70%). The primary objective of the Phase I part of this study is to determine the MTD

	of the proposed regimen defined as the dose of carfilzomib added to standard R-ICE chemotherapy. The basic design of the Phase I trial will utilize a standard 3+3 dose-finding scheme which is a special case of the A + B design described by Lin and Shih (2001). Rationale behind the design is nested in the assumption that both the probabilities of toxicity and efficacious response are continuous monotonic non-decreasing functions of the dose. The objective of the Phase Ib component is examination of the efficacy and toxicity of the addition of carfilzomib to R-ICE at the MTD dose. The primary objective of the Phase Ib component is to assess the efficacy of the study treatment arm as compared to historical control. The primary efficacy endpoint will be best overall response rate (PR+CR) within following 3 cycles of C-R-ICE therapy (as demonstrated by whole body PET-CT and dedicated CT scans following Cycle 3 of therapy). **PK/PD Analysis:** A pharmacologically-driven PK/PD model will be developed to elucidate the temporal relationship between carfilzomib concentrations and the time course of proteasome inhibition, along with temporal relationships with thrombocytopenia and neutropenia. This model will allow assessment of the dose and schedule at which carfilzomib facilitates maximal proteasome inhibition and thus lead to an increase response rate, while modulating the degree of severe toxicities. In addition, the effect of concomitant administration of rituximab, ifosfamide, carboplatin, and etoposide will be incorporated into the model to assess their potential effects on efficacy and/or toxicity.

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INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Participant Name: (Network sites use participant initials): _____

Medical Record No.: (Network sites use participant initials) _____

Title: Phase I Study of Carfilzomib Plus Rituximab Plus Ifosfamide Plus Carboplatin Plus Etoposide (C-R-ICE) in Patients with Relapsed/Refractory Diffuse Large B-cell Lymphoma (DLBCL)

INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "YES or "N/A" for patient enrollment.**	**Date**
☐	☐	☐	1. Histological confirmation of relapsed/refractory CD20 positive diffuse large B-cell lymphoma. Network sites must submit slides to Roswell Park for Central Review and confirmation.	
☐	☐	☐	2. Ann Arbor Stage I – Stage IV DLBCL at the time of relapsed/refractory disease to be eligible	
☐	☐	☐	3. Measurable or assessable disease is required. Measurable tumor size (at least one node measuring 2.25 cm^2 in bidimensional measurement) per CT scan, other radiological study, and/or physical exam.	
☐	☐	☐	4. Patients must have received at least 1 prior rituximab-based immunochemotherapy (e.g., R-CHOP, R-EPOCH, etc.).	
☐	☐	☐	5. ≥ 2 weeks since major surgery.	
☐	☐	☐	6. Patients must not have any significant toxicity associated with prior surgery, radiation therapy, chemotherapy, or immunotherapy, per PI discretion	
☐	☐	☐	7. Age ≥ 18 years and ≤ 75 years of age.	
☐	☐	☐	8. Life expectancy ≥ 3 months.	
☐	☐	☐	9. Karnofsky Score (KS) ≥ 50 (**Appendix B**).	
☐	☐	☐	10. Adequate hepatic function, with serum ALT/AST ≤ 3.5 times the upper limit of normal in the absence of a history of Gilbert's disease (or pattern consistent with Gilbert's), within 14 days prior to starting therapy.	
☐	☐	☐	11. Adequate bone marrow reserve (If patient has cytopenias due to bone marrow involvement, these requirements are not applicable): • Absolute neutrophil count (ANC) ≥ 1.0 × 10^9/L within 14 days prior to starting therapy. • Hemoglobin ≥ 8 g/dL (80 g/L) within 14 days prior to starting therapy (subjects may be receiving red blood cell [RBC] transfusions in accordance with institutional guidelines). • Platelet count ≥ 50 × 10^9/L (≥ 20 × 10^9/L if lymphoma involvement in the pre-treatment bone marrow is found) within 14 days prior to	

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INCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "YES or "N/A" for patient enrollment.**	**Date**
			starting therapy.	
☐	☐	☐	12. Patients must have a serum creatinine of ≤ 1.5 mg/dL; if creatinine > 1.5 mg/dL creatinine clearance must be > 60 mL/min within 7 days prior to treatment either measured or calculated using a standard Cockcroft and Gault formula. Refer to **Appendix C**.	
☐	☐	☐	13. Written informed consent in accordance with federal, local, and institutional guidelines.	
☐	☐	☐	14. Females of childbearing potential (FCBP) must agree to ongoing pregnancy testing and to practice contraception. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.	
☐	☐	☐	15. Male subjects must agree to practice contraception.	
☐	☐	☐	16. No known hypersensitivity to murine products.	
☐	☐	☐	17. Patients must have normal baseline cardiac function based upon echocardiogram or gated blood pool scan (MUGA) with an ejection fraction ≥ 50%.	
☐	☐	☐	18. Patients who test positive for HepC Ab are eligible provided all of the following criteria are met: a) bilirubin ≤ 2 x upper limit of normal; b) ALT/AST ≤ 3 x upper limit of normal; and c) clinical evaluation to rule out cirrhosis.	
☐	☐	☐	19. Specific guidelines will be follow regarding inclusion of relapsed/refractory DLBCL based on Hepatitis B serological testing as follow: • HBsAg negative, HBcAb negative, HBsAb positive patients are eligible. • Patients who test positive for HBsAg are ineligible (regardless of other Hepatitis B serologies). • Patients with HBsAg negative, but HBcAb positive (regardless of HBsAb status) should have a HBV DNA testing done and protocol eligibility determined as follow: If HBV DNA is positive, the subject will be excluded from the study. If HBV DNA is negative, the subject may be included but must undergo at least every 2 months HBV DNA PCR testing from the start of treatment throughout the duration the treatment course.	

Investigator Signature: _____ **Date:** _____

INVESTIGATOR STUDY ELIGIBILITY VERIFICATION FORM

Participant Name: (Network sites use participant initials)_____

Medical Record No.: (Network sites use participant initials) _____

Title: Phase I/Ib Study of Carfilzomib Plus Rituximab Plus Ifosfamide Plus Carboplatin Plus Etoposide (C-R-ICE) in Patients with Relapsed/Refractory Diffuse Large B-Cell Lymphoma (DLBCL)

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "NO" or "N/A" for patient enrollment**	**Date**
☐	☐	☐	1. Patients with NHL other than DLBCL; including "transformed" DLBCL.	
☐	☐	☐	2. Known to be seropositive for HIV. An HIV test is not required for entry on this protocol, but is required if the patient is perceived to be at risk.	
☐	☐	☐	3. Positive serology for HBV defined as a positive test for HBsAg. In addition, if negative for HBsAg but HBcAb positive (regardless of HBsAb status), a HepB DNA test will be performed and if positive the subject will be excluded.	
☐	☐	☐	4. Patients with symptomatic brain involvement.	
☐	☐	☐	5. Peripheral neuropathy of Grade 2 or greater severity as defined by the National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Patients with Grade 2 or higher (NCI-CTC) neuropathy.	
☐	☐	☐	6. Myocardial infarct within 6 months before enrollment, New York Heart Association (NYHA) Class II or greater heart failure, uncontrolled angina, severe uncontrolled ventricular arrhythmias, clinically significant pericardial disease, or electrocardiographic evidence of acute ischemia.	
☐	☐	☐	7. Uncontrolled intercurrent illness including, but not limited to, active infection, poorly controlled hypertension, diabetes mellitus or other serious medical or psychiatric conditions that could interfere with adherence to or completion of this study.	
☐	☐	☐	8. Pregnant or breastfeeding.	
☐	☐	☐	9. Patient has received other investigational drugs within4weeks before enrollment.	
☐	☐	☐	10. Chemotherapy within 3 weeks of the first scheduled study treatment.	
☐	☐	☐	11. Less than 2-year disease free from another primary malignancy (other than squamous or basal cell carcinoma of the skin, "in-situ" carcinoma of the cervix or breast, superficial bladder carcinoma, or previously treated localized prostate cancer with normal PSA levels). Patients are not	

EXCLUSION CRITERIA				
Yes	**No**	**N/A**	**All answers must be "NO" or "N/A" for patient enrollment**	**Date**
			considered to have a "currently active" malignancy if they have completed anti-cancer therapy, are considered by their physician to be at less than 30% risk of relapse and at least 2 years have lapsed.	
☐	☐	☐	12. Major surgery, other than diagnostic surgery, within 2 weeks.	
☐	☐	☐	13. Known history of allergy to Captisol® (a cyclodextrin derivative used to solubilize carfilzomib).	
☐	☐	☐	14. Medical condition requiring chronic use of high dose systemic corticosteroids (i.e., doses of prednisone higher than 10 mg/day or equivalent).	
☐	☐	☐	15. Prior HDC-ASCT.	
☐	☐	☐	16. Active CNS disease defined as symptomatic meningeal lymphoma or known CNS parenchymal lymphoma. A lumbar puncture demonstrating DLBCL at the time of registration to this study is not exclusion for study enrollment.	

Patient meets all entry criteria: ☐ **Yes** ☐ **No**

If "NO", do not enroll patient in study.

Investigator Signature: _____ **Date:** _____

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APPENDICES

1. BACKGROUND

In 2012 there have been approximately 70,130 new non-Hodgkin's lymphoma (NHL) cases and 18,940 deaths in the United States despite available treatment.[5] The most common type of B-cell NHL is diffuse large B-cell lymphoma (DLBCL), which has an aggressive clinical course. Diffuse large B-cell lymphoma accounts for approximately 31% of those diagnosed. State of the art treatment for diffuse large B-cell lymphoma depends on the stage of lymphoma with a realistic goal of curing these patients with induction therapy. Standard treatment typically consists of immunochemotherapy combining monoclonal antibody and combination chemotherapy. Radiation therapy may be utilized for CNS, bulky or refractory disease in some cases.

DLBCL can be divided into subgroups with distinct biological characteristics and prognosis utilizing gene expression profiling (GEP) analysis.[2-4] Initial GEP studies have identified 4 subgroups of primary DLBCL patients with different biology and clinical outcomes: germinal center B-cell-like (GCB) activated B-cell-like (ABC), primary mediastinal lymphoma (PML) and type 3 DLBCL.[3,4]

GCB, ABC and PML demonstrate significant differences in terms of prognosis, disease free survival and overall survival following systemic chemotherapy or (more recently) chemo-immunotherapy.[2,3,5]

GCB-DLBCL appears to be derived at the post-germinal state, primarily driven by deregulation of apoptosis by Bcl-6 and has an excellent response to rituximab-based chemo-immunotherapy regimens. ABC-DLBCL is driven by high levels of NFκB activity that is associated with a poor outcome despite chemo-immunotherapy. Finally, PML shares GEP signatures similar to those patients with classical Hodgkin's Lymphomas (HL) and has a good prognosis when compared to other DLBCL subtypes. Treatment-related toxicities (i.e., secondary malignancies, organ dysfunction, cytopenias, etc.) continue to be a significant problem being addressed in clinical trials.

There is a growing need to further characterize resistant DLBCL cells at the molecular level in an attempt to: identify biomarkers predictive of response to salvage therapies; better understand the mechanisms associated with acquired resistance to immunochemotherapy; and identify and develop novel therapeutic strategies against novel targets and/or pathways.

1.1. Front-Line Therapy of Patients with DLBCL

Unless contraindicated by significant and pre-existing co-morbid conditions, the treatment of DLBCL should include the use of rituximab and an anthracycline-based multiagent combination chemotherapy aimed to achieve a durable complete remission (CR) (i.e., cure). The initial treatment is subsequently tailored according to both clinical (e.g., stage) and tumor factors (e.g., Ki-67 expression, presence and type of chromosomal translocations, etc.). In general, the initial frontline management of DLBCL can be divided according to disease stage in 2 groups: localized and advanced stage.

1.1.1. Multidisciplinary Management of Early Stage DLBCL

Approximately 25% of DLBCL cases present as early stage. Localized DLBCL is defined as Ann Arbor Stage I or non-bulky Stage II disease and the management of such patients typically consists of an abbreviated course of combined systemic chemo-immunotherapy (e.g., R-CHOP) following by involved field radiation therapy (IF-XRT).[6,7]

In 1998 the Southwestern Oncology Group (SWOG) study 8736 randomly assigned 401 patients with non-bulky Stage I or Stage II NHL aggressive B-cell lymphoma, to receive either 3 cycles of chemotherapy consisting of cyclophosphamide, vincristine, doxorubicin and prednisone (CHOP) followed by IF-XRT at 40 cGy to 50 cGy versus 8 cycles of CHOP chemotherapy alone.[8] Patients managed with combined chemotherapy followed by IF-XRT had a better overall survival (OS) (77% and 64%, respectively, $p = 0.03$) and progression-free survival (PFS) (80% and 72%, respectively, $p = 0.02$) than patients treated with 8 cycles of CHOP alone after five years of follow-up.

The use of monoclonal antibodies, particularly rituximab, has changed the treatment paradigm of patients with B-cell NHL including DLBCL. Rituximab is a monoclonal antibody targeting the CD20 antigen present in normal and the majority of malignant B-cells. The mechanisms by which rituximab elucidates its anti-tumor activity includes: antibody-dependent cellular cytotoxicity (ADCC), complement-mediated cytotoxicity (CMC) and activation of intracellular pathways leading to direct apoptosis. Pre-clinical models had demonstrated that rituximab potentiates the effect of several chemotherapeutic agents in B-cell lymphoma.[9] In contrast to what has been observed in low-grade lymphomas, rituximab monotherapy has limited activity in DLBCL.[10] On the other hand, the addition of rituximab to standard doses of chemotherapy in DLBCL results in improved clinical outcomes without significant additional toxicity.

Few studies addressing the role of rituximab in the management of early stage DLBCL had been conducted. The addition of rituximab to combined modality treatment in DLBCL was evaluated in a SWOG and reported by Perky *et al.* The SWOG conducted a phase II clinical trial evaluating the addition of rituximab to an abbreviated course of CHOP chemotherapy followed by IF-XRT. The study enrolled 60 patients with limited stage DLBCL.[11] The PFS and OS were 88% and 92%, respectively and were considered to be superior to historical (chemotherapy-IF-XRT) controls.[11] The current literature supports the addition of rituximab to standard chemotherapy (e.g. R-CHOP) in the management of early stage DLBCL. The length of therapy and the utilization of radiotherapy continue to be a subject of ongoing debate.

1.1.2. Approach of Advanced-Stage DLBCL

The use of systemic chemotherapy to successfully eradicate disseminated DLBCL was first described in the early 1970s.[12,13] After these original reports, CHOP (given every 21 days, i.e., CHOP-21) regimen became the backbone of the standard of care for aggressive lymphomas in the US. Multiple attempts to improve survival in DLBCL patients led investigators to evaluate more intensive regimens.[14] Fisher *et al.* on behalf of the SWOG reported the results of a large clinical trial conducted in US. The study compared CHOP-21 chemotherapy with three intense chemotherapy regimens: M-BACOD (methotrexate, bleomycin, doxorubicin, cyclophosphamide, vincristine and prednisone), MACOP-B (methotrexate, doxorubicin, cyclophosphamide,

vincristine, prednisone and bleomycin,) and ProMACE/CytaBOM (cyclophosphamide, doxorubicin, etoposide, cytarabine, bleomycin, vincristine and prednisone). The study failed to demonstrate an improvement in outcome in the "intense" regimens compared to CHOP-21; "intense" regimens were associated with more toxicity than CHOP-21. After a follow up of 6 years the OS and PFS was not statistically significant different between the chemotherapeutic regimens tested and therefore CHOP-21 was chosen as the standard-of-care.[14]

The landmark study validating the addition of rituximab to CHOP chemotherapy was conducted by GELA. The GELA group enrolled patients with newly diagnosed aggressive B-cell lymphomas over the age of 60 years and randomized them to receive either 8 cycles of CHOP versus rituximab plus CHOP at 21 day intervals.[15] The study included patients with Stage I to Stage IV DLBCL, 59% had 3 or more IPI score risk factors and 80% had Ann Arbor Stage III or Stage IV disease. The addition of rituximab to CHOP chemotherapy resulted in higher response rates than CHOP alone (83% vs. 69%, respectively, $p = 0.005$). The PFS and OS at the interim analysis (after 18 month period of follow up) were significantly better in the R-CHOP arm ($p < 0.001$ and $p = 0.007$, respectively). A long term analysis of the study after 5 years of follow up confirmed the efficacy of combining rituximab with systemic chemotherapy in terms of PFS (54% vs. 30%) and OS (64.5% vs. 45%) ($p < 0.0001$ and $p = 0.0004$, respectively) A subset analysis demonstrated that, the benefit of rituximab added to CHOP chemotherapy was observed across all the different risk categories as determined by IPI risk stratification.[15,16]

A second study conducted primarily in the United States, was aimed to validate the results from the GELA study. Preliminary data from the Eastern Cooperative Oncology Group (ECOG) Study 4944 in previously untreated elderly DLBCL randomized to R-CHOP vs. CHOP, and in responders to subsequent observation versus rituximab-maintenance showed that the addition of rituximab to chemotherapy either during induction treatment or during maintenance improved the time to progression (TTP) as compared to patients treated with CHOP chemotherapy alone.[17]

Similar findings were found in younger patients with DLBCL with 0 to 1 IPI score risk factors. Pfreundschuh *et al.* reported a statistical significant benefit by adding rituximab to CHOP or CHOP-like chemotherapy in 824 patients with DLBCL in terms of PFS and OS. After a follow up period of 3-years, patients randomized to receive chemotherapy and rituximab had higher EFS (79% vs. 59%, $p < 0.0001$), and had an increased OS (93% vs. 84%; $p = 0.0001$) compared to patients assigned to chemotherapy alone.[18]

The benefit of adding rituximab to high-intermediate and high-risk DLBCL patients younger than 60 years of age has not been formally studied. The use of rituximab in combination with CHOP chemotherapy in this group of patients has been extrapolated from the results of the GELA and MInT studies. While the addition of rituximab to standard doses of CHOP chemotherapy have improved the outcomes of patients with DLBCL, a significant number of patients fail to achieve an objective response or relapse after initial response which stresses the need for the development of novel therapeutic strategies.

In the front-line setting, three alternative strategies have been or are currently being evaluated in DLBCL patients; the concept of dose-dense rituximab (i.e., 12 doses of rituximab delivered concomitantly with 6 cycles of CHOP-14); targeting of the ubiquitin proteasome systems with bortezomib as a way to potentiate the anti-tumor activity of chemo-immunotherapy; and the use

of high dose-chemotherapy and autologous stem cell support (HDC-ASCS) in first remission (especially for those patients with high risk-disease).

1.2. New Challenges in the Management of R-CHOP Relapsed/Refractory DLBCL

The PARMA study established the role of HDC-ASCS as consolidation treatment for patients with relapsed/refractory DLBCL responding to second-line chemotherapy.[19] There is a vast number of regimens used in the treatment of patients with relapsed/refractory DLBCL and are largely chosen by their relative lack of "cross-resistance" to front-line drugs. The goal of salvage regimens is to achieve maximum tumor cytoreduction in preparation for HDC-ASCS. In general, when selecting the optimal salvage regimen, regimens with high response rates (especially high CR rates), tolerable hematological and non-hematological toxicity, and those that allow effective autologous peripheral stem cell collection (PBSC) will be considered.

As rituximab changed the treatment paradigm of patients with DLBCL, it has been postulated that the current subset of patients with refractory or relapsed DLBCL represents a different patient population to the one studied in the pre-rituximab era. Several investigators questioned if response to second-line chemotherapy or if the outcome from HDC-ASCS in patients with relapsed/refractory DLBCL previously treated with "R"-CHOP to historical controls was different as compared to patients treated prior to the rituximab era.

Dr. Martin, on behalf of the Grupo Espanol de Linfomas/Transplante Autologo de Medula Osea (GEL/TAMO), reported results from a retrospective analysis in the outcome of patients with DLBCL evaluating the influence of the inclusion of upfront rituximab to rituximab plus etoposide, methylprednisolone, cytarabine and cisplatin (ESHAP) as salvage therapy.[20] Martin and colleagues studied 163 consecutive patients with relapsed/refractory DLBCL who received R-ESHAP as second line therapy, 94 patients were previously treated with rituximab-chemotherapy (R+ group) in the front line setting and 69 patients received only chemotherapy alone (R- group). Response rates were higher in patients who were not previously exposed to rituximab in a univariate analysis (but not in a multivariate analysis). The overall response and complete response rates to R-ESHAP were 67% and 37% in the treatment group versus 81% and 56% for patients in the R-group ($p = 0.045$, $p = 0.015$, respectively).[20] In addition, the PFS and OS at 3 years were significantly higher for the patients in the R- group (57% and 64%) as compared to those patients in the R+ group (38% and 17% [$p < 0.0001$ and $p = 0.0005$], respectively). Of note: the same percentage of patients in both groups underwent subsequent HDC-ASCS. In a multivariate analysis, prior exposure to rituximab was found to be a prognostic indicator of worse PFS and OS.[20] The results of this retrospective study suggests that DLBCL patients who relapse or fail to respond to rituximab-chemotherapy in the front-line setting develop more resistant disease and represent an emerging challenge for clinicians. It also stresses the need to further study and define the mechanisms and factors associated with chemoimmunotherapy resistance.

The need to develop novel salvage chemotherapy regimens after rituximab-CHOP failures was further demonstrated by the results of the Phase III collaborative trial in relapsed aggressive lymphoma (CORAL) study. This study was aimed to define the most appropriate salvage chemotherapy regimen and the role (if any) of rituximab maintenance after HDC-ASCS. In this

study, DLBCL patients with relapsed/refractory disease after CHOP or R-CHOP were randomized to receive 3 cycles of R-ICE or R-DHAP followed by conditioning chemotherapy with carmustine, etoposide, cytarabine and melphalan (BEAM) and ASCS. Subsequently, patients were randomized to rituximab maintenance or observation. The study enrolled a total of 396 patients. The investigators demonstrated that factors affecting event-free survival (EFS), include: secondary line age-adjusted international prognostic index (aaIPI) of 0 - 1 (39% vs. 56% $p = 0.0084$), relapse < 12 months after completion of first-line therapy (36% vs. 68%, $p < 0.001$) and prior rituximab exposure in the frontline setting (34% vs. 66%, $p < 0.001$)[21]. While no significant differences in terms of response rates were observed between patients treated with R-ICE or R-DHAP, the overall response rate (ORR) to salvage chemotherapy was lower among patients previously treated with R-CHOP (51%) when compared to those patients treated with upfront CHOP (83%). Moreover, the CR rate in R-CHOP pre-treated patients to either R-ICE or R-DHAP was only 38%.[21,22]

In summary, the incorporation of upfront rituximab to standard doses of CHOP (R-CHOP-21) has resulted in an improved clinical outcome when compared to CHOP-21 alone in patients with DLBCL. While the clinical benefit of adding rituximab to CHOP or CHOP-like chemotherapy in the front line treatment of DLBCL is beyond dispute, it has also caused a new challenge for the development of salvage therapies with improved therapeutic outcomes in these patients. In other words, R-CHOP has not only improved the survival in patients with DLBCL but also has modified the biology and response to subsequent therapy for those patients with refractory or relapsed DLBCL.

A group of Roswell Park investigators have demonstrated that the ubiquitin-proteasome system (UPS) plays an important role in the acquirement of resistance to rituximab and chemotherapy agents in B-cell lymphoma.[23,24] It is anticipated that targeting the UPS with novel proteasome inhibitors such as carfilzomib may result in higher overall and complete remission rates and improved outcomes following high-dose chemotherapy with autologous stem cell transplantation (HDC-ASCT) in relapsed/refractory DLBCL patients.

1.3. The Ubiquitin-Proteasome Pathway in the Development, Maintenance and Progression of Cancer

Previously, rituximab-chemotherapy resistant cells have an abnormal ubiquitin-proteasome system have been noted. The discovery and functional characterization of the ubiquitin-proteasome pathway as the major system for extra-liposomal protein degradation has delineated its importance for regulating the selective proteolysis of key regulatory proteins.[25] A significant number of proteins that regulate cell cycle, apoptosis, cell proliferation and differentiation are now known to undergo processing and functional limitation by entering the ubiquitin-proteasome pathway. The degradation of intracellular proteins by the proteasome is a multi-step process in which proteins must be “flagged” for recognition and subsequently destroyed by the 26S proteasome. Targeting of proteins for degradation occurs by a process known as ubiquitination and consists of the covalent attachment of multiple monomers of the polypeptide, ubiquitin, to a given protein. The ubiquitination of proteins is controlled by three classes of enzymes 1) ubiquitin-activating enzymes (E1), 2) ubiquitin-conjugating enzymes (E2), and 3) ubiquitin-protein ligases (E3).[26,27] Rituximab-chemotherapy-resistant cell lines, developed in

Roswell Park laboratory, express elevated levels of genes encoding several components of the UPS system suggesting enhanced proteasome-mediated protein turnover.[28]

Cytotoxic effects of proteasome inhibition have long been appreciated, but only more recently has this phenomenon been taken advantage of clinically. In addition to a central role in degrading misfolded or unfolded proteins, the proteasome has also been implicated in DNA repair and cell cycle control.[29-31] The mechanisms by which proteasome inhibition kills malignant cells have not been fully defined, but likely includes indirect effects on the function or generation of multiple gene products, particularly those regulated by the NF-kB pathway. Activity of the transcription factor nuclear factor-κB (NF-κB) is regulated by proteasomal degradation of its cytosolic binding partner IκB.[32] One prevalent theory suggests that active NF-κB found in many tumor cells supports their survival and that removal of this survival signal via bortezomib-induced stabilization of IκB favors cell death.[33] Indeed, when specific inhibitors of NF-κB are used, expression of several anti-apoptotic proteins are reduced and tumor cells undergo apoptosis.[33,34] Furthermore, direct inhibition of NF-κB with a specific inhibitor or bortezomib sensitized rituximab/chemotherapy-resistant B-NHL cell lines to the cytotoxic activity of rituximab.[35] Clinically, bortezomib was found to improve the activity of standard chemotherapeutic agents (EPOCH) in relapsed/refractory DLBCL patients with activated B-cell (i.e., ABC-like) gene expression profiles which are associated with activation of NF-κB, suggesting that bortezomib is capable of re-sensitizing NF-κB-driven DLBCL cells to chemotherapy.[36] These data do not rule out the distinct possibility that multiple NF-κB *independent* mechanisms may also contribute to the cytotoxic activity of bortezomib and other more potent proteasome inhibitors such as carfilzomib.

New treatment strategies utilizing novel agents that can potentially overcome the observed resistance pathways in therapy-resistant lymphoma cells are desperately needed. In this regard, proteasome inhibitor-based salvage regimens for refractory DLBCL hold great promise. The current clinical study proposes to define anti-tumor activity of carfilzomib, a novel proteasome inhibitor when combined with R-ICE in patients with relapsed/refractory DLBCL.

1.4. Carfilzomib

Carfilzomib (PR-171) is a tetrapeptide ketoepoxide-based inhibitor specific for the chymotrypsin-like active site of the 20S proteasome. Carfilzomib is structurally and mechanistically distinct from the dipeptide boronic acid proteasome inhibitor bortezomib (Velcade®). In addition, when measured against a broad panel of proteases including metalol, aspartyl, and serine proteases, carfilzomib demonstrated less reactivity against non-proteasomal proteases when compared to bortezomib.[37,38]

1.4.1. Toxicology Studies

In the initial Good Laboratory Practice (GLP)-compliant toxicity studies done by Onyx Pharmaceuticals, carfilzomib was administered to rats and monkeys as 2 complete 2-week cycles of QD x 5 for 5 days with nine days rest.[39,40] Administration to rats at 12 mg/m^2, the severely toxic dose in 10% of animals (STD_{10}), caused > 90% proteasome inhibition in red blood cells 1 hour after dosing. Overall, stronger inhibition of the proteasome and longer duration of

inhibition was tolerated with carfilzomib compared with bortezomib. Daily administration of bortezomib at anti-tumor doses is not tolerated in animals, and therefore daily bortezomib has not been given in the clinic. A dose-dependent decrease in proteasome activity was demonstrated in animals, and equivalent levels of proteasome inhibition were achieved with administration of carfilzomib as either an intravenous (IV) push or an IV infusion. The dose-limiting toxicities (DLTs) of carfilzomib in both the rat and monkey 28-day GLP toxicity studies included toxicity to the gastrointestinal tract, bone marrow, pulmonary, and cardiovascular systems. No behavioral or histopathological signs of neurotoxicity were observed, and carfilzomib does not cross the blood-brain barrier.

In 6-month rat and 9-month chronic toxicity studies, carfilzomib was administered on Days 1, 2, 8, 9, 15, and 16 of a 28-day cycle, mimicking the active anti-tumor regimen being used in ongoing Phase II studies in myeloma and solid tumors.[40] Tolerability was excellent, with no evidence of peripheral (or central) neurotoxicity, including neuropathology, observed, even at high doses. This is in stark contrast to that observed with bortezomib.[41]

DLTs included effects on the gastrointestinal, renal, pulmonary, and cardiovascular systems and appeared to be related to Cmax effects. Of note, neutropenia was not observed; rather, transient neutrophilia was seen following acute dosing. Renal, cardiovascular and gastrointestinal toxicities were similar to those observed with bortezomib. Finally, cyclical thrombocytopenia, likely due to inhibition of platelet budding from megakaryocytes, was similar to that seen with bortezomib. Proteasome inhibition in the blood in excess of 90% was achievable at well-tolerated doses, which contrasts with approximately 70% proteasome inhibition achievable with bortezomib at its maximum tolerated dose (MTD). In summary, these animal toxicity studies support the tolerability of carfilzomib in clinical studies, even on intensive dosing schedules and at doses achieving proteasome inhibition in excess of what can be achieved with bortezomib at its MTD on a less intensive schedule.

1.4.2. Carfilzomib Pre-Clinical Antitumor Activity

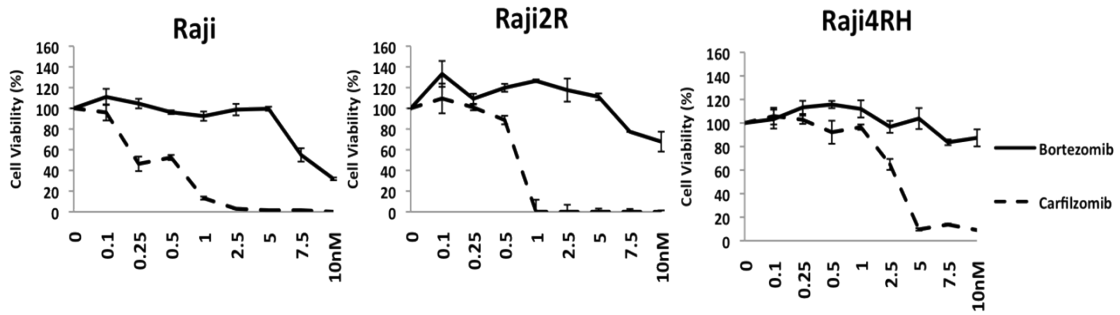
Based upon the results of *in vitro* and *in vivo* studies, it is anticipated that the more intense and longer duration of proteasome inhibition that can be achieved with carfilzomib will result in enhanced anti-tumor activity relative to bortezomib. Continuous (72 hours) exposure to carfilzomib is associated with potent cytotoxic and pro-apoptotic activity across a broad panel of tumor-derived cell lines in culture.[37,42] Incubation of hematologic tumor cell lines with carfilzomib for as little as 1 hour leads to rapid inhibition of proteasome activity followed by accumulation of polyubiquitinated proteins and induction of apoptotic cell death. Carfilzomib has also been demonstrated to be cytotoxic in bortezomib-resistant tumor cell lines.[37,42]

The anti-tumor efficacy of carfilzomib has been tested in immunocompromised mice implanted with a variety of tumor cell lines. In a human colorectal adenocarcinoma model HT-29, administration of carfilzomib on a twice-weekly Day 1, Day 2 schedule resulted in significant reduction in tumor size and was superior to both a twice-weekly Day 1, Day 4 schedule using the same dose of carfilzomib, and a once-weekly dosing schedule using twice the dose level. Bortezomib at its MTD has no activity in this xenograft model using the standard Day 1, Day 4 schedule.[37]

A group of Roswell Park investigators (i.e., Department of Medicine Lymphoma/Myeloma Laboratory at Roswell Park) evaluated the biological activity of carfilzomib alone or in combination with chemotherapeutic agents in rituximab-chemotherapy sensitive (RSCL) or resistant (RRCL) cell lines and in tumor cells derived from B-cell lymphoma patients. B-cell lymphoma cells were exposed to escalating doses of carfilzomib or bortezomib (1 nM - 7.5 nM) alone or in combination with doxorubicin, cisplatin, carboplatin, etoposide, paclitaxel, or vincristine for 24, 48, and 72 hours. Cell viability was determined by cell titer glow luminescent assay and cell cycle was analyzed by FASCan DNA methodology. In addition, Western blots were performed using cell lysates from carfilzomib, bortezomib or control-treated cells to detect PARP-cleavage and/or changes in Bcl-2 family members.

Research has found that carfilzomib was more active than bortezomib and exhibited dose-dependent and time-dependent cytotoxicity against RSCL or RRCL. A 10-fold concentration difference between carfilzomib and bortezomib activity was found. See **Figure 1.**

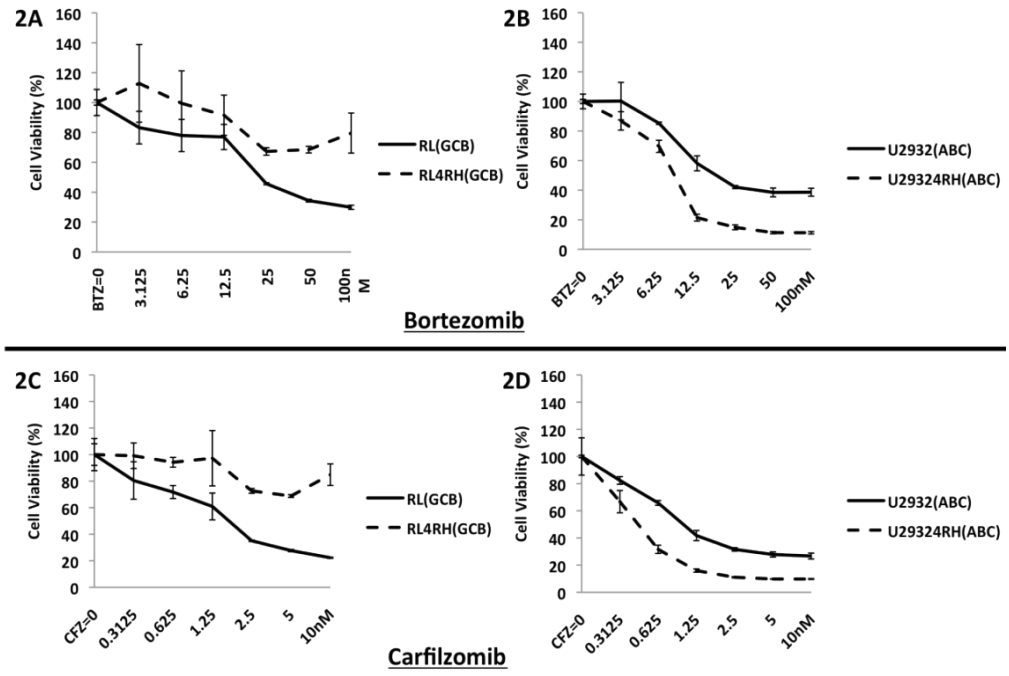
Figure 1. Dose-Dependent Effects of Carfilzomib and Bortezomib Shown in Raji Family Cells



Raji and Raji therapy-resistant cell lines Raji2R and Raji4RH were exposed to carfilzomib or bortezomib at the indicated concentrations in RPMI in 386 well plates for 48 hour and cell viability was assessed by cell titer Glo assay. The data are presented as the mean (+ S.D.) of 3 independent experiments

In addition, the anti-tumor activity of carfilzomib in GCB or ABC DLBCL cell lines will be evaluated. Of interest, in both types of cell lines carfilzomib was more potent than bortezomib in inducing cell death and a decrease in cell proliferation (**Figure 2**).

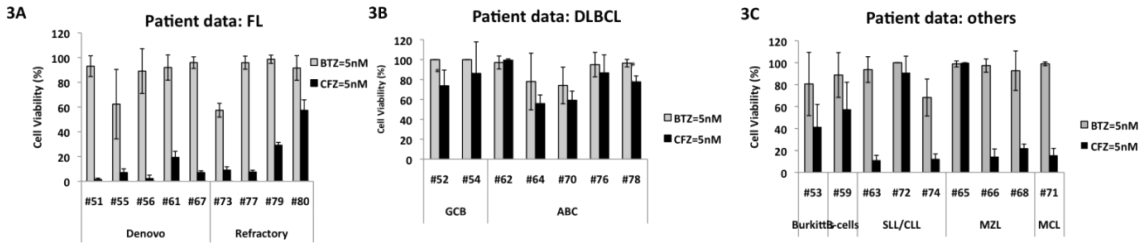
Figure 2. Effects of Carfilzomib and Bortezomib Observed in ABC and GCB Sensitive and Resistant Cells



Different effects of carfilzomib and bortezomib are observed in ABC and GCB sensitive and resistant cells. 2A, 2B, dose-dependent effects of bortezomib were determined on cell viability in RL, RL4RH, U2932 and U29324RH. Cells were exposed to bortezomib at the indicated concentrations for 48 hours. Cell viability was determined by cell titer Glo assay. 2C, 2D, smaller doses of carfilzomib were used (10% of bortezomib concentrations) to treat cells; cell viability was calculated after 48 hours. Each dataset presents the mean ± SD of 3 independent experiments.

Subsequently, the activity of carfilzomib in a more clinically relevant setting has been evaluated. Primary tumor cells were isolated from various patients with B-cell lymphoma (n = 25) and exposed to either carfilzomib or bortezomib. In general, carfilzomib was more active than bortezomib in primary tumor cells isolated from B-cell lymphoma patients (**Figure 3**).

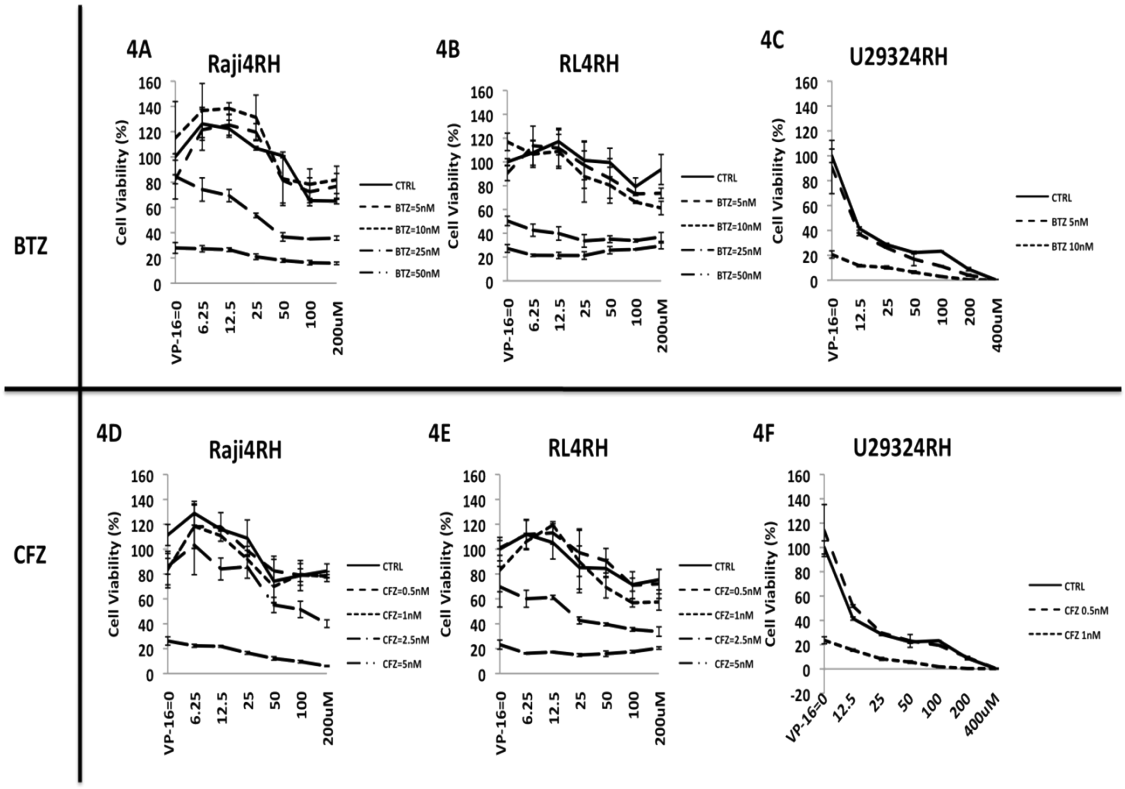
Figure 3. Various Subtypes of Lymphoma Patient Samples Sensitive to Carfilzomib



Lymphoma cells derived from patients with follicular lymphoma (FL) -3A, diffuse large B-cell lymphoma (DLBCL) -3B; and others (Burkitt's lymphoma, marginal zone lymphoma, mantle cell lymphoma, CLL) 3C were exposed to either carfilzomib 5 nM or bortezomib 5 nM. The percentage of cell viability was evaluated by Cell titer Glo assay after 48 hours of incubation. Asterisks (*) indicate a significant ($p < 0.01$) difference between carfilzomib treatment patient sample and bortezomib group.

In addition, in vitro exposure of RRCL or RSCL to carfilzomib resulted in G2/M phase cell cycle arrest. In addition, carfilzomib exposure resulted in the upregulation of Bak and Noxa levels and subsequent PARP cleavage in RRCL (Data not show). Finally, carfilzomib demonstrated the ability to overcome resistance to chemotherapy in RRCL (**Figure 4** and **Figure 5**).

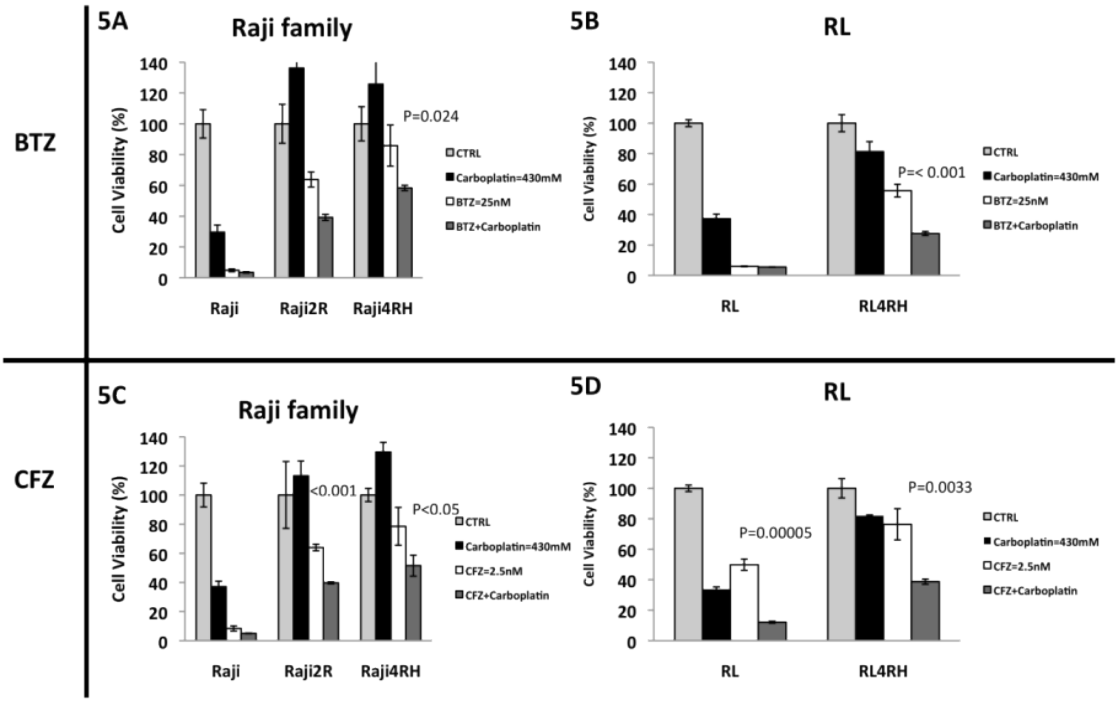
Figure 4. Carfilzomib Overcomes Rituximab-Resistance in Combination with VP-16



Raji4RH, RL4RH and U29324RH were pre-exposed to bortezomib (5 nM and 10 nM) (4A, 4B, 4C) or carfilzomib (0.5 nM, 1 nM, 2.5 nM and 5 nM) (4D, 4E, 4F) for 24 hours and subsequently exposed to various doses of VP-16 for another 48 hours. Cell viability was measured and calculated. Carfilzomib can achieve the same killing capacity as bortezomib, but at a much low dose (10% dosage of bortezomib).

In vitro exposure of rituximab-sensitive or -resistant cell lines to proteasome inhibition augmented the cytotoxic activity of several chemotherapy agents proposed to be used in this clinical trial (e.g., etoposide and carboplatin). (**Figure 4** and **Figure 5**).

Figure 5. Carfilzomib Potentiates Carboplatin Anti-Tumor Activity in Both Raji and RL Sensitive and Resistant cells



Raji and RL sensitive and resistant cells were pre-exposed to bortezomib (25 nM) (5A, 5B) or carfilzomib (2.5 nM) (5C, 5D) followed with or without carboplatin (430 mM) for another 48 hours. There are statistically significant differences among Raji and RL sensitive and resistant cells treated with the combination of proteasome inhibitor and carboplatin ($p < 0.05$). Carfilzomib can achieve approximately the same killing capacity as bortezomib, but at a much low dose (2.5 nM vs. 25 nM).

1.4.3. Phase I Experience with Carfilzomib as a Monotherapy

The results of a Phase I clinical trial, PX-171-002, testing carfilzomib in subjects with relapsed/refractory hematologic malignancies, was recently reported.[43] During the dose escalation portion of the trial, 36 subjects received carfilzomib on Days 1, 2, 8, 9, 15, and 16 of a 28-day cycle. Subjects with multiple myeloma (MM), non-Hodgkin's lymphoma (NHL), Waldenström's macroglobulinemia, and Hodgkin's lymphoma (HL) were enrolled in the study.

No dose limiting toxicities (DLTs) were observed in the initial seven cohorts (doses ranged from 1.2 mg/m^2 to 15 mg/m^2) of 3 subjects each. At the 20 mg/m^2 dose level, 1 of 8 patients had a Grade 3 renal failure at Cycle 1, Day 2 which was considered possibly related to study drug and lasted for 6 days. The patient continued on study for the remainder of Cycle 1 before having disease progression. At the 27 mg/m^2 dose level, 1 of 6 subjects experienced a DLT during Cycle 1, consisting of severe hypoxia with pulmonary infiltrates following Day 2 of dosing. In subjects where the 27 mg/m^2 dose was efficacious, a "first dose effect" was seen that included a

constellation of findings that appeared to be the clinical sequelae of rapid tumor lysis syndrome (TLS) and/or cytokine release. This effect was notable for fever, chills, and/or rigors occurring during the evening following the first day of infusion. On the second day, three of 5 subjects with multiple myeloma experienced an increase in creatinine to Grade 2 (including the subject with the DLT). This elevation was rapidly reversible and all three subjects were rechallenged with carfilzomib without recurrence of the events. Interestingly, all 3 subjects had a rapid decline in serum and/or urine M-protein levels; 2 subjects achieved a PR and the third subject achieved a minimal response (MR). There were no consistent changes in potassium, calcium, phosphorous, or uric acid levels although some increases in LDH and other markers of tumor lysis were noted. Because of the possible TLS and reversible creatinine elevations, hydration and very-low dose dexamethasone prophylaxis were instituted in subsequent studies and have essentially eliminated clinically significant TLS/creatinine elevations and the other "first-dose" effects.

Hematologic toxicities were primarily mild or moderate. The thrombocytopenia reported with carfilzomib is cyclical and similar to that reported with bortezomib. The cause and kinetics of the thrombocytopenia following treatment are different from those of standard cytotoxic agents. To maximize the likely benefit of carfilzomib, subjects with thrombocytopenia should be supported as clinically indicated rather than having treatment reduced due to thrombocytopenia.

Of the 36 evaluable patients enrolled in PX-171-002, 20 had MM.[43] Four MM patients achieved a partial response (PR), 1 of 2 at the 15 mg/m^2 dose, 1 of 6 at the 20 mg/m^2 dose, and two of five at the 27 mg/m^2 dose. The responses have been rapid in onset, beginning in some subjects after 1 – 2 doses. The duration of response (DOR) ranged from 134 to 392 days. The minimal effective dose was 15 mg/m^2 wherein > 80% proteasome inhibition in peripheral blood and mononuclear cells was observed 1 hour after dosing. The median number of prior therapies for subjects on this trial was five, and responses were seen in subjects who had relapsed from (including some refractory to) bortezomib and/or immunomodulatory agents. Stable disease also occurred in 4 NHL and 5 MM subjects, with subjects on therapy for up to 409 days. Such prolonged therapy, at "full" twice-weekly doses, is not possible with bortezomib. These results led to the initiation of 2 Phase II studies.

1.4.4. Phase II Experience with Carfilzomib as a Monotherapy

Two Phase II clinical studies are ongoing with carfilzomib in MM patients, PX-171-003-A0 (N = 46) in relapsed and refractory MM and PX-171-004 (N = 39) in relapsed MM. In both studies, patients were dosed with 20 mg/m^2 on Days 1, 2, 8, 9, 15, and 16 on a 28 day schedule. In these studies there were 4 cases of suspected or documented TLS prior to institution of the prophylaxis guidelines. Since these guidelines were implemented, no further cases of TLS have been reported including in > 350 additional patients with relapsed or refractory MM treated in ongoing Phase II studies. In both studies, the most common adverse events were fatigue, anemia, thrombocytopenia (primarily cyclical), gastrointestinal, and dyspnea. Almost all were Grade 1 or Grade 2. There were reported cases of increased in serum creatinine that were primarily < Grade 2 and were transient, rapidly reversible, and non-cumulative. A very low rate of treatment-emergent peripheral neuropathy, 2.2% Grade 3/4, was observed in PX-171-003-A0 despite the fact that 78% of patients had Grade 1/2 neuropathy upon study entry.[44]

The response rate in PX-171-003-A0 was 18% PR, 7% MR and 41% SD in these patients that entered the study with progressive disease and were refractory to their most recent therapy, often including bortezomib and/or an immunomodulatory drug (usually lenalidomide). The median time to progression on the PX-171-003-A0 study was 5.1 months with a DOR of 7.4 months (mean follow up of 7.6 months).[44]

A "stepped up" dosing schedule, referred to as 20/27 mg/m^2, has subsequently been incorporated into the PX-171-003 study (referred to as PX-171-003-A1) in order to maximize the clinical benefit of carfilzomib. Patients receive 20 mg/m^2 for the first cycle and 27 mg/m^2 thereafter. The study completed enrollment of 266 patients by the end of 2009 and may form the basis for an accelerated approval NDA filing in the future. To date, this dosing schedule has been well tolerated.[43] An independent Safety Oversight Group (SOG) evaluated the safety data from the 40 of 250 patients to be enrolled on the 20/27 schedules and agreed that the trial should proceed without modification. No cases of TLS were observed and rates of BUN and creatinine elevation dropped sharply, with Grade 3/4 renal impairment dropping to 2.2% in A1 (from 15% in A0), most likely due to hydration and very low dose dexamethasone. The other most common adverse events were similar to the A0 portion of the study. Treatment-emergent peripheral neuropathy remains low on this portion of the study with 15% Grade 1/2 and one (0.7%) Grade 3/4 events reported to date on PX-171-003-A1.[44] In addition, anemia rates in the PX-171-003-A1 (higher dose) were lower than those reported in the PX-171-003–A0 portion of the study, possibly indicating that the higher dose of carfilzomib is achieving better clearing of neoplastic cells in the bone marrow allowing superior normal marrow reconstitution. Rates of thrombocytopenia and neutropenia were similar in the 2 cohorts, with Grade 3 neutropenia in approximately 5% without any Grade 4 neutropenia to date.[44]

In PX-171-004, a first cohort of patients received 20 mg/m^2. The subset of patients (N = 54) that had not seen bortezomib had an ORR of 46% (2% CR, 9% VGPR and 35% PR), while the bortezomib treated patients (N = 33) had an ORR of 18% (3% CR, 3% VGPR and 12% PR).[45] The median TTP was 7.6 and 5.3 months in these 2 groups, respectively. Thus, carfilzomib can induce very high levels of response in patients who have not previously been treated with bortezomib and, even in bortezomib-treated patients, substantial anti-tumor activity is observed. Of note, disease control (PR + MR + SD) was achieved in approximately 65% of patients with progressive MM entering the study. Patients on these studies have been treated for > 12 cycles with good tolerability and no cumulative toxicity (e.g., bone marrow, severe fatigue, or neuropathy) have not been observed.

The protocol was amended to allow patients to increase to 27 mg/m^2 in Cycle 2 or later based on tolerability, similar to that used in PX-171-003–A1. Further information about the Phase II studies is presented in the Investigator's Brochure.

1.4.5. Experience with Carfilzomib in Combination with Lenalidomide and Dexamethasone

PX-171-006 is an ongoing Phase Ib study in patients with relapsed multiple myeloma in which carfilzomib is administered in combination with lenalidomide (Revlimid®) and dexamethasone. "Low-dose" dexamethasone 40 mg/day is given on Days 1, 8, 15, and 22 in all cases.

Carfilzomib is administered IV on Days 1, 2, 8, 9, 15, and 16; lenalidomide is administered PO on Days 1 through 21. Enrollment has closed in this study, and no MTD was reached. The maximum per protocol doses of carfilzomib (27 mg/m^2) with lenalidomide 25 mg and low dose dexamethasone are being used.[46] After 8 patients tolerated these doses well, an additional 44 patients were enrolled in an "expansion" cohort at this level, and this regimen is being taken into Phase III in study PX-171-009.

To date, 40 patients were treated in Cohorts 1 - 6 and 44 in the Cohort 6 expansion. 27/32 patients in cohorts 1 - 5 are evaluable for safety and 29/32 for response. Patients were heavily pre-treated; 72% received prior BTZ and 87.5% received prior LEN or thalidomide (Thal). 47% of patients were refractory to their last therapy (typically lenalidomide + high dose dexamethasone; > 84% of patients had a history of neuropathy with 67% BTZ- or Thal-related. No treatment emergent fatigue, neuropathy, or thrombotic events ≥ Grade 3 were observed. Hematological AEs ≥ Grade 3 (thrombocytopenia [n = 6], anemia [n = 4], and neutropenia [n = 6]) were reversible. 4 patients had drug-related SAEs as follows: transient Grade 3 sinus bradycardia, Grade 3 upper respiratory tract infection, febrile neutropenia, and Grade 3 diarrhea + Grade 3 urinary infection. ORR and CBR for the 29 evaluable patients are 59% and 72%, respectively. Response data is shown in the table below. Initial responses improved with continued therapy, (up to 18 cycles). Median duration of response has not been reached (median follow-up 5.2 months). No dose-limiting toxicities or deaths attributed to study treatment have been observed. Several patients have completed the study (in the lower dose cohorts) after 18 cycles and are continuing in an extension study. Updated efficacy data are presented in the following table:

Table 1. CRd: Cohorts 1–5 (Carfilzomib: 15 to 20 mg/m^2; LEN: 10 to 25 mg)

Response	Relapsed (n = 16)	Refractory (n = 13)	Overall (n = 29)
≥ CR/nCR	5 (31)	1 (8)	6 (21)
≥ VGPR	7 (44)	4 (31)	11 (38)
≥ PR	9 (56)	8 (62)	17 (59)
≥ MR	11 (67)	10 (77)	21 (72)

Together, these results suggest that carfilzomib, lenalidomide, and low-dose dexamethasone (CRd) in combination are active and well tolerated and that there are no significant overlapping toxicities (in the dose ranges tested). Importantly, lenalidomide-associated neutropenia and thrombocytopenia do not appear to be exacerbated by concurrent treatment with carfilzomib, even up to 27 mg/m^2, suggesting that carfilzomib will combine well with other anti-cancer agents.

2. RATIONALE

Current standard salvage chemotherapy regimens (e.g., R-ICE, R-DHAP, etc.) used prior to HDC-ASCR in patients with relapsed/refractory DLBCL yield suboptimal outcomes. The ORR and CR response rate following salvage therapy with R-ICE or RDHAP is 63% and 38%

respectively. Moreover, the 3-year progression-free survival of relapsed/refractory DLBCL patients initially treated with R-CHOP and then salvage R-ICE or R-DHAP and HDC-ASCT is only 30%. The incorporation of active novel agents into the management of relapsed/refractory DLBCL is needed in an attempt to improve clinical outcomes.

A group of Roswell Park investigators has demonstrated that the ubiquitin-proteasome system plays an important role in the development of rituximab-chemotherapy resistance in B-cell lymphoma. Moreover, targeting the proteasome system with pharmacological inhibitors has resulted in significant anti-tumor activity in relapsed/refractory DLBCL. While the combination of bortezomib and systemic chemotherapy is currently being studied in patients with relapsed/refractory DLBCL, bortezomib is limited by its toxicity profile. Carfilzomib is a more potent and better tolerated proteasome inhibitor when compared to bortezomib. The study is seeking to determine the maximum tolerable doses of carfilzomib when administered in combination with the R-ICE chemotherapy regimen in relapsed/refractory DLBCL patients who are ASCT-eligible.

2.1. Dose Rationale

Preliminary data suggest that carfilzomib as a single agent can produce substantial response rates in myeloma and B-cell lymphoma subjects across a variety of dosing cohorts. Responses were seen over a wide therapeutic window, from 15 mg/m^2 to 27 mg/m^2. Maximum proteasome inhibition was seen at doses 11 mg/m^2 and higher in whole blood samples taken 1 hour after the first dose. The final analysis of the human pharmacokinetic (PK) data is ongoing but appears to be rapid and similar to the results from the animal studies. Carfilzomib is rapidly cleared from plasma with an elimination half-life of < 60 minutes at the 20 mg/m^2 dose. Large, single arm studies of the 27 mg/m^2 dose are ongoing and suggest that this dose is very well tolerated with patients being treated for > 10 cycles without cumulative toxicities.

By the end of 2009, 269 patients with relapsed and refractory multiple myeloma have been enrolled in the PX-171-003-A1 study. The goal of dose escalating to 27 mg/m^2 beginning with Cycle 2 is to improve ORR, DOR, and TTP. In multiple preclinical studies, the tolerability of carfilzomib in rats has been shown to be significantly higher when administered as a 30 minutes infusion as compared to a rapid IV bolus. Toxicities observed with IV bolus injection of carfilzomib *above the MTD* at a dose of 48 mg/m^2 include evidence of prerenal azotemia (transient increases in BUN > creatinine) as well as lethargy, piloerection, dyspnea, and gastrointestinal bleeding. Notably, death occurred in approximately 50% of animals at 48 mg/m^2 when carfilzomib was given as a bolus. Administration of the same dose (48 mg/m^2) as a 30 minutes continuous infusion was well tolerated, with no changes in BUN and creatinine and substantially reduced signs of lethargy, piloerection, or dyspnea. Moreover, all animals in the infusion treatment groups survived. The only toxicity observed following infusion of carfilzomib for 30 minutes was gastrointestinal bleeding. The reduced toxicity seen with dosing by infusion may reflect the reduced C_{max} of carfilzomib vs that with bolus dosing.[47] Inhibition of the pharmacological target of carfilzomib (the chymotrypsin-like activity of the proteasome) was equivalent in the bolus and infusion treatment groups.

In the clinic, the MTD of carfilzomib has not been reached in the multiple myeloma (MM) setting, particularly when administered as a 30-minute infusion. 27 mg/m^2 of carfilzomib (bolus administration over 2 - 10 minutes) is well tolerated in MM patients overall and can be tolerated for > 12 cycles in late stage MM patients with substantial comorbidities.

A Phase I dose escalation study (PX-171-007) of single agent carfilzomib administered is ongoing and over 65 patients with solid tumors had received therapy in the initial Phase II portion of the study at 36 mg/m^2 (bolus administration over 2 - 10 minutes). A review of the tolerability of 36 mg/m^2 carfilzomib in these patients indicates that this regimen was very well tolerated with only one DLT (fatigue) and an overall adverse event profile similar to that seen with the 27 mg/m^2 carfilzomib experience with bolus dosing (see IB for details). Three patients completed > 12 cycles of therapy at 36 mg/m^2 with no evidence of cumulative toxicity. There were no significant DLTs observed; the majority of discontinuations on the study were due to progressive disease. Because of the long-term tolerability carfilzomib, the Phase Ib portion of this study was reopened, and a separate arm for multiple myeloma was added.

In the PX-171-007 trial, more recently patients have been treated with carfilzomib given as a 30-minute infusion in order to potentially minimize Cmax-related infusion events. The protocol was amended and does of 20/36 (20 mg/m^2 given on Day 1 and Day 2 of Cycle 1 only; followed by 36 mg/m^2 for all subsequent doses), 20/45, 20/56 mg/m^2 and so forth are being investigated. Doses of 20/56 mg/m^2 are currently being given in 2 separate cohorts of patients with advanced MM and advanced solid tumors; the lower doses were well tolerated. Preliminary tolerability information at this dose level (20/56 mg/m^2) indicated that it is reasonably well tolerated with minimal infusion reactions. In some cases at 20/56 mg/m^2, dexamethasone was increased from 4 mg/dose to 8 mg with the 56 mg/m^2 doses in order to reduce fevers and hypotension. As of March 20, 2010, 7 patients have received 20/56 mg/m^2 and are tolerating it. Patients with advanced, refractory MM being treated at 36 mg/m^2 and 45 mg/m^2 have shown very good tolerability (> 6 months in some cases) with documented minimal and partial responses in these heavily pretreated patients. These data indicate that carfilzomib 30-minute infusion can be given at very high levels, with > 95% inhibition of blood proteasome levels achievable and with (at least) acute tolerability. All protocols using ≥ 36 mg/m^2 carfilzomib are now administering the drug as a 30-minute infusion.

In addition to the above observations, a Phase I study of carfilzomib in patients with relapsed and refractory multiple myeloma was reported in abstract form at the 2009 American Society of Hematology meeting which demonstrated that carfilzomib can be safely administered to patients with substantial renal impairment (CrCL < 30, including patients on dialysis) without dose adjustment.[48] These data indicate that carfilzomib does not exacerbate underlying renal dysfunction, and confirm the "pre-renal" etiology of the BUN/creatinine elevations observed with IV bolus carfilzomib.

3. OBJECTIVES

3.1. Phase I Primary Objectives

- To estimate the maximum tolerated dose (MTD) and examine the dose-limiting toxicities of carfilzomib when administered in combination with rituximab, ifosfamide, carboplatin, and etoposide (C-R-ICE) in patients with relapsed/refractory DLBCL.
- To assess the toxicity of dose regimen using the CTEP NCI Common Terminology Criteria for Adverse Events (CTCAE Version 4.0).

3.2. Phase Ib Primary Objectives (Expansion cohort)

- To evaluate the safety of carfilzomib (given at maximum tolerated dose [MTD] as determined in Phase I of this study) in combination with R-ICE salvage therapy in relapsed/refractory DLBCL patients.
- To achieve an overall response rate (CR and PR) of 70% after 3 cycles of C-R-ICE in patients between the ages of 18 to 75 with relapsed/refractory CD20-positive DLBCL previously treated with rituximab-based immunochemotherapy (e.g., R-CHOP, R-EPOCH, R-HyperCVAD, etc.) induction.

3.3. Secondary Objectives

- To determine the feasibility of successful mobilization of autologous stem cells (i.e., minimum of 2 x 10^6 CD34+ cells/kg should be collected) to be used for ASCT (if applicable).
- To determine toxicities associated with C-R-ICE salvage therapy.
- To determine the TTP, PFS, and OS followed by ASCT (if applicable); Disease-free survival in CR patients.
- To determine the pharmacokinetics/pharmacodynamics relationship between carfilzomib's degree of proteasome inhibition and response rate along with the time course of thrombocytopenia.
- To study differences in clinical outcomes between GCB and non-GCB relapsed/refractory DLBCL following therapy with carfilzomib and R-ICE.

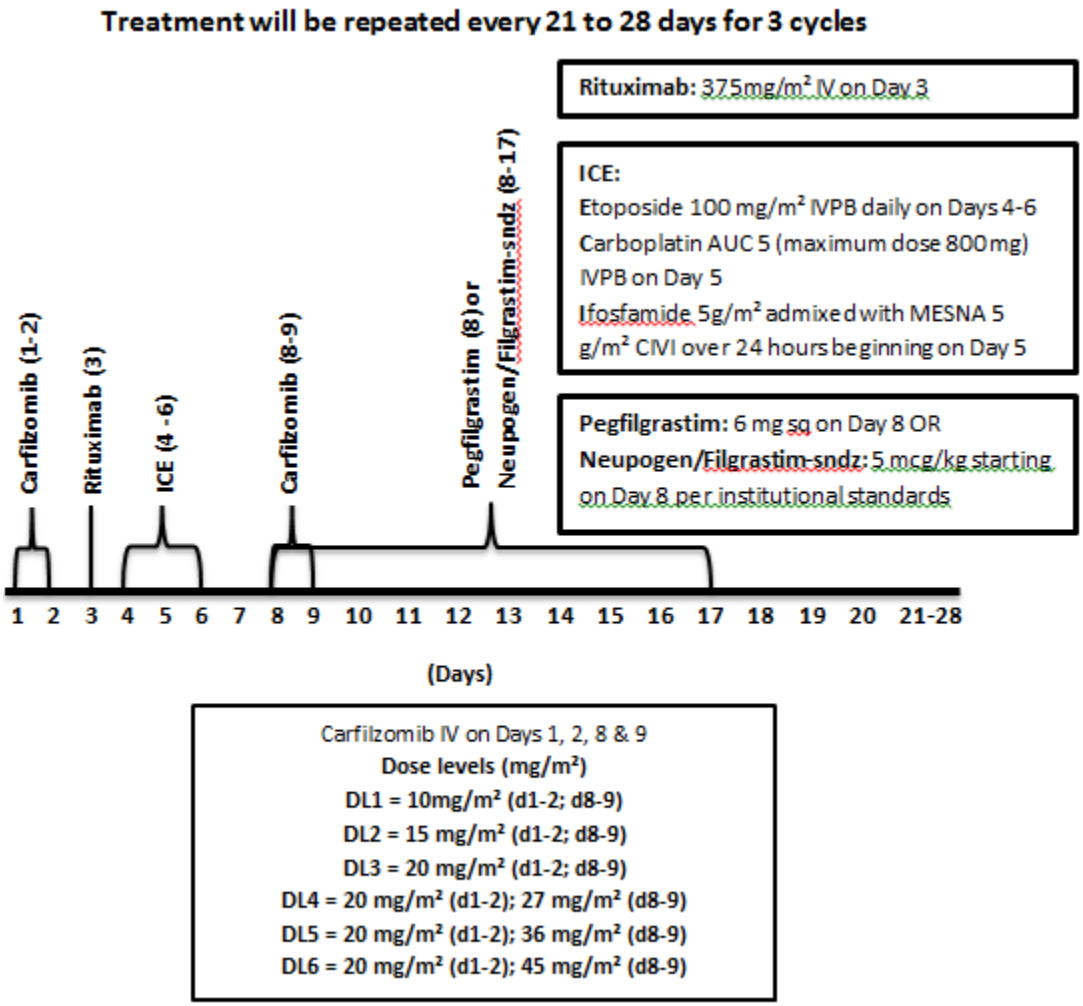
Correlative translational research studies to include: phenotypic/genotypic analysis and functional activity (i.e., ADCC and CMC) of patient's peripheral blood mononuclear "effector" cells (PBMC), as well as ex vivo analysis of sensitivity of primary tumor cells to various combinations of carfilzomib versus Bortezomib +/- rituximab;, enzymatic assay for chymotrypsin-like activity to determine the degree of proteasome inhibition in primary DLBCL patient samples and patient PBMC specimens; explorative analysis to identify potential factors predictive of response to therapy will be performed (as part of an NIH-supported R01 grant: R01 CA136907-0A1; PI: Francisco J. Hernandez-Ilizaliturri, MD).

4. METHODOLOGY

4.1. Study Design

This is an open-label, non-randomized, multicenter, dose-escalation Phase I, followed by Phase Ib expansion study of carfilzomib in combination with R-ICE regimen in relapsed/refractory DLBCL patients. Based on the results of the Phase I portion of the study, the Phase Ib dose will be at Dose Level 6. The study schema is depicted in **Figure 6**.

Figure 6. Treatment Plan Schema



Subjects will be treated on an inpatient and outpatient basis. All subjects will meet the inclusion and exclusion criteria summarized in **Section 5.1** and **Section 5.2** prior to enrollment in this study.

4.2. Target Accrual and Study Duration

A maximum of 21 patients for Phase I and 9 patients for the Phase Ib expansion part of this trial will be enrolled at Roswell Park and Network Sites (For Phase Ib only). The number of subjects required is a function of a currently unknown dose-toxicity relationship. Accrual is expected to take up to 10 years.

5. SUBJECT SELECTION

5.1. Inclusion Criteria

To be included in this study, subjects must meet the following criteria:

5.1.1 Disease Specific

- Histological confirmation of relapsed/refractory CD20 positive diffuse large B-cell lymphoma. Network sites must submit slides to Roswell Park for Central Review and confirmation.
- Ann Arbor Stage I – Stage IV DLBCL at the time of relapsed/refractory disease to be eligible.
- Measurable or assessable disease is required. Measurable tumor size (at least one node measuring 2.25 cm^2 in bidimensional measurement) per CT scan, other radiological study, and/or physical exam.

5.1.2 Prior Treatment

- Patients must have received at least 1 prior rituximab-based immunochemotherapy (e.g., R-CHOP, R-EPOCH, etc.).
- ≥ 2 weeks since major surgery.
- Patients must not have any significant toxicity associated with prior surgery, radiation therapy, chemotherapy, or immunotherapy, per PI discretion

5.1.3 Demographic

- Age ≥ 18 years and ≤ 75 years of age.
- Life expectancy ≥ 3 months.
- Karnofsky Score (KS) ≥50 (**Appendix B**).

5.1.4 Laboratory

- Adequate hepatic function, with serum ALT/AST ≤ 3.5 times the upper limit of normal in the absence of a history of Gilbert's disease (or pattern consistent with Gilbert's), within 14 days prior to starting therapy.

- Adequate bone marrow reserve (If patient has cytopenias due to bone marrow involvement, these requirements are not applicable):
    - Absolute neutrophil count (ANC) $\geq 1.0 \times 10^9$/L within 14 days prior to starting therapy.
    - Hemoglobin $\geq$ 8 g/dL (80 g/L) within 14 days prior to starting therapy (subjects may be receiving red blood cell [RBC] transfusions in accordance with institutional guidelines).
    - Platelet count $\geq 50 \times 10^9$/L ($\geq 20 \times 10^9$/L if lymphoma involvement in the pre-treatment bone marrow is found) within 14 days prior to starting therapy
- Patients must have a serum creatinine of $\leq$ 1.5 mg/dL; if creatinine > 1.5 mg/dL creatinine clearance must be > 60 mL/min within 7 days prior to treatment either measured or calculated using a standard Cockcroft and Gault formula. Refer to **Appendix C**.

5.1.5 Medical/Ethical

- Written informed consent in accordance with federal, local, and institutional guidelines.
- Females of childbearing potential (FCBP) must agree to ongoing pregnancy testing and to practice contraception. Should a woman become pregnant or suspect she is pregnant while she or her partner is participating in this study, she should inform her treating physician immediately.
- Male subjects must agree to practice contraception.
- No known hypersensitivity to murine products.
- Patients must have normal baseline cardiac function based upon echocardiogram or gated blood pool scan (MUGA) with an ejection fraction $\geq$ 50%.
- Patients who test positive for Hepatitis C Antibody are eligible provided all of the following criteria are met: a) bilirubin $\leq$ 2 x upper limit of normal; b) ALT/AST $\leq$ 3 x upper limit of normal; and c) clinical evaluation to rule out cirrhosis.
- Specific guidelines will be follow regarding inclusion of relapsed/refractory DLBCL based on Hepatitis B serological testing as follow:
    - HBsAg negative, HBcAb negative, HBsAb positive patients are eligible.
    - Patients who test positive for HBsAg are ineligible (regardless of other Hepatitis B serologies).
    - Patients with HBsAg negative, but HBcAb positive (regardless of HBsAb status) should have a HBV DNA testing done and protocol eligibility determined as follow:
        - If HBV DNA is positive, the subject will be excluded from the study.

- If HBV DNA is negative, the subject may be included but must undergo at least every 2 months HBV DNA PCR testing from the start of treatment throughout the duration the treatment course.

5.2. Inclusion of Women and Minorities

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

5.3. Exclusion Criteria

Subjects will be excluded from this study for the following:

- Patients with NHL other than DLBCL; including "transformed" DLBCL.
- Known to be seropositive for HIV. An HIV test is not required for entry on this protocol, but is required if the patient is perceived to be at risk.
- Positive serology for HBV defined as a positive test for HBsAg. In addition, if negative for HBsAg but HBcAb positive (regardless of HBsAb status), a HepB DNA test will be performed and if positive the subject will be excluded.
- Patients with symptomatic brain involvement.
- Peripheral neuropathy of Grade 2 or greater severity as defined by the National Cancer Institute's (NCI) Common Terminology Criteria for Adverse Events (CTCAE) Version 4.0. Patients with Grade 2 or higher (NCI-CTC) neuropathy.
- Myocardial infarct within 6 months before enrollment, New York Heart Association (NYHA) Class II or greater heart failure, uncontrolled angina, severe uncontrolled ventricular arrhythmias, clinically significant pericardial disease, or electrocardiographic evidence of acute ischemia.
- Uncontrolled intercurrent illness including, but not limited to, active infection, poorly controlled hypertension, diabetes mellitus or other serious medical or psychiatric conditions that could interfere with adherence to or completion of this study.
- Pregnant or breastfeeding.
- Patient has received other investigational drugs within 4 weeks before enrollment.
- Chemotherapy within3 weeks of the first scheduled study treatment.
- Less than 2-year disease free from another primary malignancy (other than squamous or basal cell carcinoma of the skin, "in-situ" carcinoma of the cervix or breast, superficial bladder carcinoma, or previously treated localized prostate cancer with normal PSA levels). Patients are not considered to have a "currently active" malignancy if they have completed anti-cancer therapy, are considered by their physician to be at less than 30% risk of relapse and at least 2 years have lapsed.
- Major surgery, other than diagnostic surgery, within 2 weeks.

- Known history of allergy to Captisol® (a cyclodextrin derivative used to solubilize carfilzomib).
- Medical condition requiring chronic use of high dose systemic corticosteroids (i.e., doses of prednisone higher than 10 mg/day or equivalent).
- Prior HDC-ASCT.
- Active CNS disease defined as symptomatic meningeal lymphoma or known CNS parenchymal lymphoma. A lumbar puncture demonstrating DLBCL at the time of registration to this study is not exclusion for study enrollment.

6. DRUG INFORMATION

6.1. Carfilzomib (Investigational Agent)

Generic Name: Carfilzomib is the generic name; formerly referred to as PR-171.

Chemical Name: (2S)-N-((S)-1-((S)-4-methyl-1-((R)-2-methyloxiran-2-yl)-1-oxopentan-2-ylcarbamoyl)-2-phenylethyl)-2-((S)-2-(2-morpholinoacetamido)-4-phenylbutanamido)-4-methylpentanamide.

Molecular Formula: C40H57N5O7

Molecular Weight: 719.9

Amgen, Inc. will supply non-commercial carfilzomib to the investigator. Carfilzomib for injection (formerly referred to as PR-171 for injection) is supplied as a lyophilized parenteral drug product in 45 mg and 60 mg single-use vials. Prior to administration, the lyophilized product is aseptically reconstituted with sterile water for injection. Upon reconstitution, carfilzomib for injection consists of 2 mg/mL carfilzomib, 100 mg/mL sulfob tylether beta-cyclodextrin (SBE-β-CD), and 1.9 mg/mL citrate buffer (pH 3 to 4).

Preparation: Remove the vial from the refrigerator just prior to use. Aseptically reconstitute each vial by slowly injecting 29 mL of sterile water for injection, directing the solution onto the inside wall of the vial to minimize foaming. Gently swirl and/or invert the vial slowly for about 1 minute, or until complete dissolution of any cake or powder occurs. Do not shake to avoid foam generation. If foaming occurs, allow the solution to rest in the vial for about 2 to 5 minutes, until foaming subsides. The reconstituted solution contains carfilzomib 2 mg/mL. After reconstitution, carfilzomib is ready for IV administration. When administering in an IV bag, withdraw the calculated dose from the vial and dilute into dextrose 5% injection 50 mL D5W bag. The reconstituted product should be a clear, colorless solution. If any discoloration or particulate matter is observed, do not use the reconstituted product.

6.1.1. Drug Shipment

Carfilzomib will be provided by Amgen, Inc. The site will document the date of receipt and condition of the shipment. The investigational pharmacist or designee will retain drug-shipment records.

6.1.2. Drug Preparation and Administration

Carfilzomib for injection is supplied as a lyophilized parenteral product in single-use vials. The lyophilized product is reconstituted with water for injection to a final carfilzomib concentration of 2 mg/mL prior to administration. The dose will be calculated using the subject's actual BSA at baseline. Subjects with a BSA > 2.2 m^2 will receive a dose based upon a 2.2 m^2 BSA.

IV hydration will be given immediately prior to carfilzomib during Cycle 1. This will consist of 250 mL to 500 mL normal saline or other appropriate IV fluid. If lactate dehydrogenase (LDH) or uric acid is elevated (and/or in subjects considered still at risk for TLS) at Cycle 2 Day 1, then the recommended IV hydration should be given additionally before each dose in Cycle 2. The goal of the hydration program is to maintain robust urine output (e.g., ≥ 2 L/day). Subjects should be monitored periodically during this period for evidence of fluid overload.

If the subject has a dedicated line for carfilzomib administration, the line must be flushed with a minimum of 20 mL of normal saline prior to and after drug administration.

Carfilzomib will be given as an IV infusion over approximately 10 minutes. For doses > 27 mg/m^2, carfilzomib should be infused over 30 minutes. The dose will be administered at a facility capable of managing hypersensitivity reactions. Subjects will remain at the clinic under observation for at least 1 hour following each dose of carfilzomib in Cycle 1 and following the dose on Cycle 2 Day 1. During these observation times, post dose IV hydration (between 250 mL and 500 mL normal saline or other appropriate IV fluid formulation) will be given. Subjects should be monitored periodically during this period for evidence of fluid overload.

6.1.3. Storage and Stability

Carfilzomib for injection is stored refrigerated at 2°C to 8°C. After addition of the appropriate amount of water for injection and vigorous mixing, the solution is administered as an IV infusion. Please note: the use of closed-system drug-transfer devices, where multiple stopper punctures in close proximity to one another can occur, is not recommended as it could result in coring.

The reconstituted solution in its original vial can be stored in a refrigerator controlled at 2°C to 8°C (recommended), or at room temperature (15°C to 30°C) until use; and the reconstituted solution should not be frozen. Reconstituted carfilzomib for injection is a clear, colorless to slightly yellow solution, and is essentially free of visible particulates.

Parenteral drug products should be visually inspected for particulate matter and discoloration prior to administration, whenever solution and container permit. If particulate matter or discoloration is observed, the reconstituted product should not be used.

6.1.4. Handling and Disposal

The Investigator or designee will be responsible for dispensing and accounting for all investigational drug provided by Amgen, Inc., exercising accepted medical and pharmaceutical practices. Study drugs must be handled as cytotoxic agents and appropriate precautions taken per the institution's environmentally safe handling procedures. All investigational drugs will be dispensed in accordance with the Investigator's prescription or written order.

Records of product lot numbers and dates received will be entered on a product accountability form. It is the Investigator's responsibility to ensure that an accurate record of investigational drug issued and returned is maintained.

Used vials (excess drug) will be destroyed according to standard practices at the site after properly accounting for the dispensing. Partially used vials of study drug will not be re-used for other subjects. Expired product and/or unused vials will be inventoried by the study monitor.

Under no circumstances will the Investigator supply investigational drug to a third party or allow the investigational drug to be used in a manner other than as directed by this protocol.

6.2. Rituximab (Rituxan®)

Rituximab is a chimeric monoclonal antibody. Rituximab binds to the CD20 antigen expressed on benign and malignant B-cells and causes cell death by complement mediated lysis, induction of apoptosis and by antibody dependent cellular cytotoxicity (ADCC).

Formulation: Rituximab is a genetically engineered, chimeric, murine/human monoclonal antibody directed against the CD20 antigen found on the surface of normal and malignant pre-B and mature B cells. The antibody is an IgG1 κ 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 2 light chains of 213 amino acids (based on cDNA analysis) and has an approximate molecular mass of 145 kD. Rituximab has a binding affinity for the CD20 antigen of approximately 8nM. Rituximab is supplied as 100 mg and 500 mg sterile, preservative-free, single-use vials.

Supplier: Commercially available and will not be provided for this study.

Preparation: DO NOT ADMINISTER AS AN INTRAVENOUS PUSH OR BOLUS. Do not infuse rituximab concomitantly with another IV solution or other IV medications. The appropriate dose is withdrawn and diluted to a final concentration of 1 - 4 mg/mL in either 0.9% sodium chloride or 5% dextrose solution. The solution is then stable at 2° to 8°C for 24 hours and at room temperature for an additional 12 hours.

Storage: Vials can be stored at 2˚C to 8˚C. They should be protected from sunlight.

Administration: The first infusion should be administered at an initial rate of 50 mg/hr. If hypersensitivity or infusion-related events do not occur, the rate may be increased by 50 mg/hr every 30 minutes up to a maximum of 400 mg/hr. Subsequent infusions may be started at 100 mg/hr and the rate increased by 100 mg/hr at every 30 minutes to a maximum of 400 mg/hr, as tolerated. Rituximab may also be infused per institutional guidelines at the discretion of the PI. Patients will be premedicated with acetaminophen 650-mg po and diphenhydramine 50 mg IV 30 minutes prior to beginning the rituximab infusion. For severe reactions, the infusion will be stopped and can be resumed at 50% of the prior rate once the reactions are treated and symptoms resolved.

Toxicity:

- **Common:** Fever, chills, fatigue, headache; less common: nausea, vomiting, rhinitis, pruritus, hypotension; rare: neutropenia, thrombocytopenia, asthenia, arthritis, vasculitis,

lupus-like syndrome, pleuritis, bronchiolitis obliterans, uveitis, optic neuritis, and skin reactions such as toxic epidermal necrolysis and pemphigus.

- **Fatal Infusion Reactions:** Severe and fatal cardiopulmonary events, including angioedema, hypoxia, pulmonary infiltrates, acute respiratory distress syndrome, myocardial infarction, and cardiogenic shock, have been reported. These severe reactions typically occurred during the first infusion with time to onset of 30 - 120 minutes.
- **Cardiac Events:** Patients with preexisting cardiac conditions, including arrhythmia and angina, have had recurrences of these cardiac events during rituximab infusions.
- **Tumor Lysis Syndrome (TLS):** TLS has been reported and is characterized in patients with a high number of circulating malignant cells ($\geq$ 25,000 μL) by rapid reduction in tumor volume, renal insufficiency, hyperkalemia, hypocalcemia, hyperuricemia, and hyperphosphatemia.
- **Renal Events:** Rituximab has been associated with severe renal toxicity including acute renal failure requiring dialysis, and in some cases has led to death. Renal toxicity has occurred in patients with high numbers of circulating malignant cells ($\geq$ 25,000/mm^2) or high tumor burden who experience tumor lysis syndrome and in patients administered concomitant cisplatin.
- **Mucocutaneous Reactions:** Severe bullous skin reactions, including fatal cases of toxic epidermal necrolysis and paraneoplastic pemphigus, have been reported in patients treated with rituximab. The onset of reaction has varied from 1 to 13 weeks following rituximab exposure.
- **Hematologic Events:** In clinical trials, Grade 3 and Grade 4 cytopenias were reported in 48% of patients treated with RITUXIMAB; 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 RITUXIMAB therapy were reported.

  In addition, there have been a limited number of post-marketing reports of prolonged pancytopenia, marrow hypoplasia, and late onset neutropenia (defined as occurring 40 days after the last dose of RITUXIMAB) in patients with hematologic malignancies. In reported cases of late onset neutropenia (NCI-CTC Grade 3 and Grade 4), the median duration of neutropenia was 10 days (range 3 to 148 days). Documented resolution of the neutropenia was described in approximately one-half of the reported cases; of those with documented recovery, approximately half received growth factor support. In the remaining cases, information on resolution was not provided. More than half of the reported cases of delayed onset neutropenia occurred in patients who had undergone prior autologous bone marrow transplantation. In an adequately designed, controlled, clinical trial, the reported incidence of NCI-CTC, Grade 3 and Grade 4 neutropenia was

higher in patients receiving RITUXIMAB in combination with fludarabine as compared to those receiving fludarabine alone (76% [39/51] vs. 39% [21/53]).

- **Infectious Events:** Rituxan induced B-cell depletion in 70% to 80% of patients with NHL and was associated with decreased serum immunoglobulins in a minority of patients; the lymphopenia lasted a median of 14 days (range, 1 - 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. Serious infectious events (Grade 3 or Grade 4), including sepsis, occurred in 2% of patients.
- **Other Serious Viral Infections:** 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.

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

  PML is a rare and demyelinating disease of the brain caused by infection with the JC virus that usually leads to death or severe disability. JC virus infection resulting in PML and death has been reported rarely in patients with hematologic malignancies receiving rituximab. The majority of these patients had received rituximab in combination with chemotherapy or as part of a hematopoietic stem cell transplant. Cases of PML resulting in death have also been reported in patients with systemic lupus erythematosus (SLE) treated with rituximab. These patients with SLE had longstanding disease, history of prior immunosuppressant therapy, and were diagnosed with PML within 12 months of their last infusion of rituximab.

  Physicians should consider PML in any patient presenting with new onset neurologic manifestations. Consultation with a neurologist, brain MRI, and lumbar puncture should be considered as clinically indicated. In patients who develop PML, rituximab should be discontinued and reductions or discontinuation of any concomitant chemotherapy or immunosuppressive therapy should be considered.
- **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.

- **Additional Safety Signals:** The following serious adverse events have been reported to occur in patients following completion of rituximab infusions: arthritis, disorders of blood vessels (vasculitis, serum sickness and lupus-like syndrome), eye disorders (uveitis and optic neuritis), lung disorders including pleuritis and scarring of the lung (bronchiolitis obliterans), that may result in fatal outcomes, and fatal cardiac failure.

6.3. Ifosfamide (Ifex®)

Supplier: Commercially available and will not be provided for this study.

Mechanism of Action: Ifosfamide is activated in the liver by microsomal enzymes and the subsequent ifosfamide mustard causes direct alkylation of DNA.

Formulation: Ifosfamide is supplied in single dose vials for constitution and administration by IV infusion. Each contains 1 gram or 3 grams of sterile ifosfamide.

Storage: The dry powder may be stored at room temperature.

Preparation: Injections are prepared by adding sterile water to the vial. The 1-gram dose is mixed with 20 mL and the 3-gram dose with 60 mL for a final concentration of 50 mg/mL.

Toxicity: Alopecia, nausea and vomiting, hematuria, gross hematuria, CNS toxicity, infection, renal dysfunction, allergic reactions and at high doses, cardiotoxicity.

6.4. Carboplatin (Paraplatin®)

Supplier: Commercially available and will not be provided for this study.

Mechanism of Action: Carboplatin binds to DNA and causes cross-linking with a non-cell cycle dependent tumor cell lysis. It inhibits DNA synthesis by altering the template via the formation of intrastrand cross-links.

Formulation: Carboplatin is available in 10 mg/mL solution in 50, 150, 450, 600 mg vials.

Storage: Unopened vials are stable for the life indicated on the insert if protected from light.

Preparation: Per Institute standard.

Toxicity: Myelosuppression, nausea, vomiting, peripheral neuropathy, ototoxicity, hepatic toxicity, electrolyte abnormalities, hypomagnesemia, hypocalcemia, and allergic reactions.

6.5. VP-16 (etoposide, VePesid®)

Supplier: Commercially available and will not be provided for this study.

Mechanism of Action: Induction of an irreversible blockade of cells in the premitotic phases of the cell cycle leading to accumulation of cells in late S or G2 phases. This mechanism is secondary to interference of the scissors-reunion reaction of the enzyme topoisomerase II.

Formulation: VP-16 injection is available in 100-mg (5-mL) sterile multiple-dose vials. The pH is 3-4. Each mL contains 20-mg etoposide, 2-mg citric acid, 30-mg benzyl alcohol, 80-mg polysorbate 80, 650-mg polyethylene glycol 300 and 30.5% alcohol.

Storage: Unopened vials of VP-16 are stable for 24 months at room temperature. Vials are diluted as recommended to a concentration of 0.2 mg/mL or 0.4 mg/mL and are stable for 96 hours and 48 hours respectively, at room temperature under normal light in both plastic and glass containers.

Preparation: As per Institute standard intravenous infusion over2 hours.

Toxicity: Leukopenia, thrombocytopenia, alopecia, nausea, vomiting, headache, fever, hypotension, anorexia, and allergic reactions.

6.6. Mesna (Mesnex®)

Supplier: Commercially available and will not be provided for this study.

Mechanism of Action: Mesna was developed as a prophylactic agent to inhibit hemorrhagic cystitis induced by ifosfamide and is analogous to the cysteine-cystine system; mesna is rapidly metabolized to mesna disulfide and acts as a free radical scavenger.

Formulation: Mesna is a sterile preservative free aqueous solution of clear, colorless appearance in clear glass vials for IV administration. Mesna injection contains 100 mg/mL Mesna, 0.25 mg/mL acetate disodium, and sodium hydroxide to maintain pH 6.5-8.5.

Storage: Diluted solutions are chemically and physically stable for 24 hours at room temperature. It is recommended that solutions be refrigerated and used within 6 hours.

Preparation: For IV administration the drug is diluted in sterile solution to make a final concentration as per Institute standard.

Toxicity: Nausea, vomiting, diarrhea

6.7. Neulasta® (Pegfilgrastim, G-CSF, Neupogen, Filgrastim-sndz and biosimilars)

Supplier: Commercially available and will not be provided for this study.

Mechanism of action: Pegfilgrastim is a human protein, which is involved in the promotion of the growth and maturation of granulocytic progenitors and the stimulation of functional activity. It is a covalent conjugate of recombinant methionyl human G-CSF (Filgrastim) and monomethoxypolyethylene glycol

Formulation: Available as a pegylated recombinant DNA product supplied as 0.6 mL prefilled syringe containing clear colorless sterile protein solution.

Storage: It can be stored at 2°C - 8°C and is stable for at least 30 months.

Toxicity: Bone pain, exacerbation of preexisting autoimmune disorders, transient and reversible changes in alkaline phosphatase, uric acid and LDH, 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

7. TREATMENT PLAN

7.1. Preparation and Administration

Treatment will be administered in an outpatient and inpatient basis. Appropriate dose modifications are described in **Section 7.4**. No other investigational or commercial drugs or therapies other that those described below may be administered with the intent to treat the patient.

7.2. General Guidelines

Prior to initiating therapy, placement of mediport or a multi-lumen indwelling intravenous catheter is required. All patients will be given allopurinol 300 mg/day PO starting at least one day prior to Treatment 1 (Day 0) and then 300 mg/day PO for Day 1 through Day 10 of the first cycle. Allopurinol length of therapy may be changed at discretion of the investigator (e.g., the presence of tumor lysis syndrome). If patient cannot receive Allopurinol for any reason, a different drug may be used for the prevention of tumor lysis syndrome at the discretion of the PI.

7.3. Criteria for Retreatment

The following laboratory parameters must be met before starting Cycles 2 and Cycle 3:

- Begin subsequent cycles no sooner than Day 22 but no later than Day 36 (If a subject is delayed beyond Day 36, contact the Principle Investigator or designee from Roswell Park)
- ANC $\geq$ 1000/µL without G-CSF
- Platelets $\geq$ 50,000/µL
- Serum creatinine $\leq$ 2.0 mg/dL
- Total bilirubin $\leq$ 2.0 mg/dL (unless due to disease involvement with DLBCL or Gilbert's syndrome).

Serum chemistry values, including creatinine, must be obtained (within 24 hours) and reviewed prior to each dose of carfilzomib. Carfilzomib must be held for subjects with a CrCL < 30 mL/min at any time during study participation. Subjects with active or suspected infection of any kind that required systemic treatment should not be dosed with carfilzomib until the infection has resolved and if being treated with anti-infective, the course of antibiotics has been completed.

7.4. Dose Modifications

7.4.1. Definition of Dose-Limiting Toxicity

For this protocol, a DLT will be defined by the occurrence of the following toxicity, related to study drug, within cycle 1 of treatment. Patients that experience a DLT will be continued on treatment, per PI discretion, at one level of dose reduction.

- Any ≥ Grade 3 non-hematologic adverse event attributed (definitely, probably, or possibly) during C-R-ICE therapy and meeting the following criteria and for hematologic toxicity see **Section 0**.

7.4.1.1 Non-Hematologic Toxicity

For non-hematologic toxicities, DLT will be defined as any CTCAE v4.0 Grade 3 or greater toxicity, except for:

- Grade 3 or Grade 4 nausea and vomiting lasting less than 72 hours after the last dose of chemotherapy with use of standard anti-emetic therapy.
- Grade 3 non-hematologic laboratory abnormalities that resolve to Grade 1 or baseline (if the patient entered the study with existing toxicity) within 14 days.
- In the event that Grade 3 or Grade 4 adverse events are obviously unrelated to study drug and thus not DLT, the principal investigator will determine whether dose escalation or cohort expansion is required.

7.4.1.2 Hematologic Toxicity

DLT for hematologic toxicity, using CTCAE v4.0, will be defined as:

- Any hematologic toxicity that causes a dose-delay of > 2 weeks (i.e., > Day + 36) of the intended next dose will also be considered at DLT.

7.4.1.3 Criteria for Dose Escalation

Dose escalation will proceed within each cohort according to the following scheme in **Table 2**.

Patients who do not have a DLT and who do not complete Cycle 1 of treatment or more if appropriate will be replaced in the study.

Management and dose modifications associated with the above AEs are outlined in **Section 7.4**.

Table 2. Dose Escalation Scheme

Number of Patients with a Dose-Limiting Toxicity at a Given Dose Level	Escalation Decision Rule
0 out of 3	Enter 3 patients at the next dose level. If there are no dose-limiting toxicities found after completing all dose levels, an additional 3 patients will be enrolled at the maximally administered dose. If this dose level is determined to be a safe dose (≤1out of 6 dose-limiting toxicities), it will then be used in the expansion phase.
≥ 2	Dose escalation will be stopped. This dose level will be declared the maximally administered dose (highest dose administered). Three additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
1 out of 3	Enter at least 3 more patients at this dose level. If 0 of these 3 patients experience a dose-limiting toxicity, proceed to the next dose level. If 1 or more of this group suffer a dose-limiting toxicity, then dose escalation is stopped, and this dose is declared the maximally administered dose. Three additional patients will be entered at the next lowest dose level if only 3 patients were treated previously at that dose.
≤ 1 out of 6 at highest dose level	This is generally the recommended Phase Ib dose. At least 6 patients must be entered at the recommended Phase Ib dose.

7.4.2 Treatment Delay Thrombocytopenia and Neutropenia

Carfilzomib is associated with thrombocytopenia. The thrombocytopenia pattern is cyclic with nadirs following the second dose each week and typically recovering prior to the initiation of the next treatment, similar to that observed with bortezomib. The severity of thrombocytopenia is related to the pretreatment platelet counts. Transfusions may be considered. The incidence of significant bleeding is < 5%. Platelet counts should be monitored at a minimum prior to each cycle of treatment and more frequently if baseline counts are > Grade 1. Severe neutropenia has been reported and may occur during treatment but is uncommon. Carfilzomib should be administered with caution to patients with ANC < 1000/mm^3 and the drug should be held for ANC < 500/mm^3. Febrile neutropenia is rare and carfilzomib should be held until the AE has resolved. In addition, patients with active infections or who are completing treatment for systemic infections should not receive carfilzomib until the infection has resolved.

7.4.2.1 Cardiac Disorders

Acute development or exacerbation of congestive heart failure and new onset of decreased left ventricular function have been reported, including reports in patients with no risk factors for decreased left ventricular ejection fraction. The incidence of heart failure events (acute pulmonary edema, cardiac failure, congestive cardiac failure, cardiogenic shock, pulmonary edema) is less than 8%. Patients with risk factors for or evidence of existing heart disease should be closely monitored throughout their treatment with carfilzomib. Carfilzomib should be held if CHF develops or appears to be exacerbated by treatment and may be resumed once the symptoms resolve. Consideration should be given to a reduction in dosage with gradual increase to full dose only if well tolerated. Ischemic heart disease and arrhythmias have also occurred. Dyspnea may occur in association with cardiac disorders. Patients who experience dyspnea

should be evaluated for the presence of associated conditions and management should be tailored to the appropriate treatment for the underlying disorder. The effect of carfilzomib on cardiac repolarization using the QTcF interval and the PK-PDn relationships shows no clear signal of any dose related effect. Serious cardiac reactions and even death may occur following the administration of carfilzomib.

7.4.2.2 Gastrointestinal Events

Carfilzomib treatment can cause nausea, vomiting, diarrhea, or constipation sometimes requiring the use of antiemetics or antidiarrheals. Fluid and electrolyte replacement should be administered to prevent dehydration. Routine premedication with antinausea or antidiarrheal agents is not required. Carfilzomib should be held if these events persist after symptomatic treatment, are Grade 3 or greater and determined by the Investigator to be related to the Carfilzomib.

7.4.2.3 Hematologic Toxicity

Typically, there is no dosages adjustment related to hematological toxicity with the R-ICE regimen as described in this study. C-R-ICE will be planned to be given on a q 21 - 28 day cycle. C-R-ICE will be postponed until neutrophil counts reach $\geq 1 \times 10^9$/L and platelet counts $\geq 50 \times 10^9$/L.

7.4.2.4 Tumor Lysis Syndrome

Suspected or documented TLS or multiorgan failures have been observed in some patients treated with carfilzomib in the Phase II studies prior to the institution of TLS prophylaxis guidelines. Patients with high tumor burden or compromised renal function may be at elevated risk, and should be closely monitored, and uric acid levels normalized prior to initiation of treatment, if appropriate. Adequate oral and IV hydration and premedication with dexamethasone 4 mg PO or IV are required prior to initiation of therapy with carfilzomib, at least in Cycle 1. If TLS occurs, carfilzomib should be held until the event has resolved. Prophylaxis should be reinstituted and the patient should be monitored carefully. Additional safety measures are described in detail in the protocols.

7.4.2.5 Infusion Reactions and Adverse Events Experienced within a Day of Dosing

True infusion reactions have been reported but are rare. Should an infusion reaction occur, treatment with glucocorticoids, IV fluids, vasopressors, oxygen, bronchodilators, and acetaminophen should be available for immediate use and instituted as medically indicated. Adverse events have been observed with carfilzomib that include fever, chills, dyspnea, and/or rigors occurring in the evening following the first day of infusion during the first cycle of therapy, which may be the clinical sequelae of rapid tumor lysis or cytokine release; this has been associated with an increase of creatinine on the second day. Cytokine release has been documented in animals treated with carfilzomib. Management guidelines were instituted which included premedication with dexamethasone (4 mg PO on Days 1, 2, 8, and 9 or equivalent) and starting patients at a 20 mg/m^2 dose for a minimum of 2 days before dose escalation. These management guidelines and stepped-up dosing were generally effective at alleviating AEs

experienced within the first day of dosing in further studies. If fevers or chills persist after the first cycle, continued premedication with dexamethasone is recommended.

7.4.2.6 Renal Impairment

Grade 1 and Grade 2 reversible increases in creatinine were reported. Renal failure has been reported less frequently. It is not known if there is a clear association between renal failure and carfilzomib. Many cases of renal failure were confounded by documented or suspected TLS, sepsis/infections, light-chain disease, and disease progression. Renal function should be monitored prior to each dose in Cycle 1 and Cycle 2 and in patients experiencing increases in creatinine ≥ Grade 2. Carfilzomib has been characterized in patients with CrCL < 15 mL/min or creatinine ≥ 2.5 mg/dL. Studies in patients with very severe renal dysfunction and those receiving hemodialysis demonstrated there were no major differences in the observed safety findings among patients with renal dysfunction compared to those with normal renal function.

7.4.2.7 Hepatic Impairment

Three cases of hepatic failure (including 2 cases that were precursors to the patients' death due to end-stage progression of their MM) and 1 case of veno-occlusive has been reported with the use of carfilzomib (refer to Carfilzomib Investigator's Brochure). Non-serious and serious (Grade 1 - 3) transaminitis have been reported infrequently. Carfilzomib dosage should be held in patients who experience a liver abnormality ≥ Grade 3. If the event resolves within 2 weeks, carfilzomib may be restarted at a reduced dose and monitored carefully. The use of carfilzomib has not been characterized in patients with hepatic impairment.

7.4.2.8 Other Conditions Delaying Treatment

If posterior reversible encephalopathy syndrome (PRES) is suspected, carfilzomib should be held. The patient should then be evaluated with MRI and for onset of visual or neurological symptoms suggestive of PRES. If PRES is confirmed, carfilzomib should be permanently discontinued. If a diagnosis of PRES is excluded, carfilzomib may be restarted per the discretion of the PI.

Carfilzomib should be held if thrombotic microangiopathy (TMA) is suspected and patient should be managed per institutional standard of care, which may include plasma exchange, if appropriate. If TMA is confirmed, carfilzomib should be permanently discontinued. If TMA is ruled out, then carfilzomib may be restarted per the discretion of the PI.

If the patient experiences a hypertensive crisis, carfilzomib should be held until the resolution of the event to the patient's baseline. Carfilzomib may then be restarted at 1 dose decrement.

7.4.2.9 Mechanisms of Action

Carfilzomib is a tetrapeptide epoxyketone proteasome inhibitor that binds selectively and irreversibly to the N-terminal threonine containing active sites of the 20S proteasome and displays little to no activity against other classes of proteases. In vitro, carfilzomib has been shown to have antiproliferative and proapoptotic activities across a range of solid and hematologic tumor cells, including cells that are resistant to bortezomib. Carfilzomib does not

induce neurite degeneration in differentiated neuronal cell lines sensitive to an equimolar concentration of bortezomib. *In vivo*, carfilzomib results in rapid and sustained inhibition of proteasome activity in blood and tissues and delays tumor growth in MM, hematologic, and solid tumor animal models, including bortezomib-insensitive human tumor xenograft models.

8 GENERAL CONCOMITANT MEDICATION AND SUPPORTIVE CARE

8.1 Safety Considerations

Based upon the experience in the Phase I and Phase II clinical studies with carfilzomib, the following observations are noted:

- A "first dose effect" has been seen, which is notable for fever, chills, rigors, and/or dyspnea occurring during the evening following the first day of infusion and an increase in creatinine on Day 2, which may be the clinical sequelae of rapid tumor lysis and/or cytokine release.
- Should a "first dose" effect occur at any point during Cycle 1 or Cycle 2, treatment with high dose glucocorticoids (e.g., methylprednisolone 50 mg - 100 mg) is recommended. In addition, intravenous fluids, vasopressors, oxygen, bronchodilators, and acetaminophen should be available for immediate use and instituted, as medically indicated.
- Dexamethasone 4 mg PO/IV will be administered prior to all carfilzomib doses.
- Acyclovir or similar should be given to all subjects with a history of herpes simplex or zoster, per institutional prophylaxis guidelines, unless contraindicated.
- CrCL changes are mostly transient, reversible, and non-cumulative. All subjects should be well hydrated. Clinically significant electrolyte abnormalities should be corrected prior to dosing with carfilzomib. Renal function must be monitored closely during treatment with carfilzomib. Serum chemistry values, including creatinine, must be obtained (within 24 hours) and reviewed prior to each dose of carfilzomib. Carfilzomib must be held for subjects with a CrCL < 30 mL/min at any time during study participation.
- Subjects with active or suspected infection of any kind that required systemic treatment should not be dosed with carfilzomib until the infection has resolved and if being treated with anti-infective, the course of antibiotics has been completed.
- Thrombocytopenia has been transient and typically resolves during the week between treatments. For platelet counts $\leq 50{,}000/mm^3$, carfilzomib dosing must be held before proceeding to the next cycle of C-R-ICE.
- Subjects should have anemia corrected in accordance with the Institutional guidelines.
- Carfilzomib treatment can cause nausea, vomiting, diarrhea, or constipation sometimes requiring the use of antiemetics or antidiarrheals. Fluid and electrolyte replacement should be administered to prevent dehydration.

8.2 Guidelines for Monitoring, Prophylaxis, and Treatment of Tumor Lysis Syndrome

- Tumor Lysis Syndrome (TLS), which may be associated with multiorgan failure, has been observed in treatment Cycle 1 and Cycle 2 in some patients with MM who have been treated with carfilzomib.

The following safety measures are mandatory for all subjects:

- In addition, subjects with high tumor burden or rapidly progressing DLBCL or compromised renal function (CrCL < 50 mL/min) should be considered to be at particularly high risk.

Hydration and Fluid Monitoring

- Oral hydration: All subjects must be well hydrated (i.e., volume replete). Begin oral hydration equal to approximately 30 mL/kg/day (approximately 6 – 8 cups of liquid per day), starting 48 hours prior to the planned first dose of carfilzomib. Compliance must be reviewed with the subject and documented by the site personnel prior to initiating treatment with carfilzomib; treatment is to be delayed or withheld if oral hydration is not deemed to be satisfactory.
- Intravenous Fluids: 250 - 500 mL of IV normal saline (or other appropriate IV fluid formulation) must be given before and after each carfilzomib dose during Cycle 1. If lactate dehydrogenase (LDH) or uric acid is elevated at Cycle 2, Day 1, then the recommended IV hydration should be repeated for Cycle 2. The goal of the hydration program is to maintain robust urine output, (e.g., ≥ 2 L/day). Subjects should be monitored periodically during this period for evidence of fluid overload.
- In subjects considered to be still at risk for TLS at completion of Cycle 1, hydration should be continued in Cycle 2, if clinically indicated. Patients in whom this program of oral and IV fluid hydration is contraindicated, e.g., due to pre-existing pulmonary, cardiac, or renal impairment, will not be eligible to participate in the clinical trial.

Laboratory Monitoring

- Appropriate chemistries, including creatinine, and complete blood counts (CBC) with platelet count should be obtained and reviewed prior to carfilzomib dosing on day 1 of each cycle. Results of laboratory studies must be reviewed and deemed acceptable prior to administering the carfilzomib dose. Subjects with laboratory abnormalities consistent with lysis of tumor cells (e.g., serum creatinine ≥ 50% increase, LDH ≥ 2-fold increase, uric acid ≥ 50% increase, phosphate ≥ 50% increase from baseline, potassium ≥ 30% increase, calcium ≥ 20% decrease) prior to dosing should not receive the scheduled dose. Subjects with such abnormalities should be re-evaluated again within the next 24 hours (or sooner, if clinically indicated) and then periodically as clinically indicated.

Clinical Monitoring

- Inform subjects of signs and symptoms that may be indicative of TLS, such as fevers, chills/rigors, dyspnea, nausea, vomiting, muscle tetany, weakness, or cramping, seizures, and decreased urine output. Advise subjects to report such symptoms immediately and seek medical attention.

Management of Tumor Lysis Syndrome

- If TLS occurs, cardiac rhythm, fluid, and serial laboratory monitoring should be instituted. Correct electrolyte abnormalities, monitor renal function and fluid balance, and administer therapeutic and supportive care, including dialysis, as clinically indicated.

8.3 Concomitant Medications

Concomitant medication is defined as any prescription or over-the-counter preparation including vitamins and supplements. Concomitant medications will not be collected at this time.

8.3.1 Required Concomitant Medications

- Female subjects of child-bearing potential must agree to use dual methods of contraception for the duration of the study and for at least 30 days after the last dose of carfilzomib. Male subjects must agree to use a barrier method of contraception for the duration of the study if sexually active with a female of child-bearing potential. Male subjects must also refrain from donating sperm for at least 90 days after the last dose of carfilzomib.
- Subjects should receive antibiotic prophylaxis with ciprofloxacin or other fluoroquinolone (or trimethoprim/sulfamethoxazole if fluoroquinoles are contraindicated). In addition, subjects should receive acyclovir or similar (famciclovir, valacyclovir) anti-varicella (anti-herpes) agent prophylaxis.
- Subjects should receive adequate granulocyte colony stimulating factor support on Day 8 of treatment.
- All subjects must receive prophylaxis with hydration as typically used for R-ICE per institutional guidelines.
- Subjects may be pretreated for nausea and vomiting with appropriate anti-emetics.
- Patients may receive transfusion support as per institutional guidelines.

8.4 Duration of Treatment

Subjects may remain on study for a maximum of 3 cycles.

8.5 Treatment Discontinuation

Upon treatment discontinuation all end of study evaluations and tests will be conducted. All subjects who discontinue due to an AE must be followed until the event resolves or stabilizes. Appropriate medical care should be provided until signs and symptoms have abated, stabilized, or until abnormal laboratory findings have returned to acceptable or pre-study limits. The final status of the AE will be reported in the subject's medical records and the appropriate eCRF.

Reasons for treatment discontinuation should be classified as follows:

- Death
- Progressive disease
- Treatment-related toxicity
- Toxicity unrelated to treatment
- Investigator judgment
    - The Investigator may withdraw a subject if, in his/her judgment, it is in the subject's best interest to do so.
- Noncompliance
- Subject voluntary withdrawal
    - A subject may withdraw from the study at any time, for any reason. If a subject discontinues treatment, an attempt should be made to obtain information regarding the reason for withdrawal.
- Early withdrawal of subject(s)
- Sponsor decision.

Subjects who are unavailable for follow-up evaluations should be classified as lost to follow-up for 1 of the following reasons:

- Lost to follow-up: For a subject to be considered lost to follow-up, the investigator must make 2 attempts to re-establish contact with the subject. The attempts to re-establish subject contact must be documented (e.g., certified letter).
- Death: Date and cause of death will be recorded for those subjects who die within 30 days after last dose of study drug (telephone contact is acceptable).

9 STUDY PROCEDURES

9.1 Subject Registration

All data management will be conducted at Roswell Park. Eligible patients will be entered on study centrally at the institute by the Study Coordinator. Following registration, patients should begin protocol treatment within 14 days or sooner if clinically necessary. Issues that would cause treatment delays should be discussed with the Principal Investigator at Roswell Park (or

designee). If a patient does not begin protocol therapy within 14 days following registration, the patient's registration on the study may be canceled. The Study Coordinator should be notified of cancellations as soon as possible.

Table 3. Study Calendar

Tests and Observations	Prior to Study[1]	Day 1 of Each Cycle[2]	At Time of Restaging[3]	Collect Stem Cell and ASCT Data (if applicable)[20]	Collect Post ASCTDay +100Data[20]	Post Treatment Follow-Up[4]
History and Physical Exam	X	X				X
Pulse, Blood Pressure	X	X				X
Height/Weight/Body Surface Area[5]	X	X				
Performance Status	X	X				X
Drug Toxicity Assessment		X				X[7]
Laboratory Studies						
CBC, Differential, Platelets	X	X[8]				X
Serum Creatinine, BUN	X	X[9]				X
Creatinine Clearance (Appendix C)	X	X[9]				
Serum Electrolytes	X	X[9]				X
Ca^{++}, Potassium	X	X[9]				X
ALT, AST, Alkaline Phosphatase, Bilirubin	X	X[9]				X
Phosphorus	X	X[9]				
LDH and Uric Acid	X	X[9]				X
EKG	X					
MUGA or ECHO	X					
HBsAg, HBcAb[10]	X					
HBV DNA, Qualitative	X[10]					
HCV Ab	X[11]					
HIV (If risk factors present)	X					
Beta-2-microglobuline (B2M)	X					
Albumin	X	X				X

Serum or Urine-βHCG (female patients)	X[12]					
Staging						
Lumbar Puncture	X[7]	X[13]				X[13]
CT Scan (Chest/Abdomen/Pelvis) and/or PET	X[6]		X			X [23]
Bone Marrow Aspiration and Biopsy	X[21]		X[14]			X[14]
Histologic Review	X[22]					X[15]
Cytogenetics (e.g., Bcl-2 or c-MYc studies)	X					X[15]
Sample Submission and Correlative Studies						
Lymph Node Biopsy	X[16,17]					
Immune Biomarker Samples	X[18]					
Pharmacokinetic /Pharmacodynamic Samples[24]		X[19]				
Adverse Events	X	X	X			X[7]

1 To be completed within28 days prior to enrollment

2 Within 48 hours prior to Day 1 of each induction treatment cycle.

3 Prior to study, at time of restaging; between Cycle 2 Day 15 to Cycle 2 Day 28 following Cycle 3 but before ASCT and at the end of treatment evaluation, then every 4 months (±2 weeks) for 1 year, then every 6 months (±2 weeks) for 2 years, then once a year (±2 weeks) for 2 years for a total of 5 years, then as clinically indicated. The follow up period will begin at the end of study evaluation visit for patients that became ineligible for transplant for any reason but did not meet criteria for disease progression. The follow up period for patients that go on to transplant will begin at the Day +100 bone marrow biopsy.

4 At least every 4 months (± 1 week) for 1 year, then every 6 months (± 1 week) for 2 years, then once a year (± 2 weeks) for 2 years for a total of 5 years from study entry, then as clinically indicated. The follow up period will start at the Day 100 bone marrow biopsy in patients that received a transplant. Patients that progress will be followed for survival status only on a yearly basis.

5 If there is a greater than 10% difference in weight from baseline, then recalculate the BSA prior to each treatment; otherwise the actual dose given does not need to change.

6 CT Scan to be completed within 28 days prior to enrollment

7 When clinically appropriate.

8 CBC with differential and platelet counts will be monitored 3 times per week in between cycles of C-R-ICE until count recovery.

9 Days 1, 2, 8, and 9

10 All patients must be screened for hepatitis B infection (HBsAg/HBcAb) before starting treatment. Patients tested positive for HBV surface antigen are ineligible. Patients tested HBsAg negative/HBcAb positive should have qualitative HBV DNA PCR testing. If HBV DNA is positive the subject is excluded. If HBV DNA is negative, subject may be included but must undergo at least every 2 months HBV DNA PCR

testing from the start of treatment throughout the duration the treatment course. Monitoring during the on treatment periods is required at least every 2 months and during follow-up at a minimum of every 2 - 3 months up to 6 months after the last dose. Prophylactic antiviral may be initiated at the discretion of the investigator.

11 All patients must be screened for HCV infection before starting treatment. Patients who test positive for HCV antibody (Ab) evaluation of LFT's and clinical evaluation to rule out cirrhosis.

12 To be completed within 7 days prior to enrollment.

13 Repeat as medically indicated if positive at baseline.

14 Document absence of disease at least once in CR if previously involved.

15 At the time of relapsed/refractory disease diagnosis.

16 If the patient is undergoing a pre-treatment fresh biopsy - collect tissue with a goal of at least 0.5 g (in saline or media (RPMI 1640) provided to pathology by Roswell Park Department of Medicine Lymphoma/Myeloma Laboratory; fresh tissue will not be collected from Network Sites

17 Repeat diagnostic lymph node/tumor biopsy if initial biopsy was not diagnostic. Do not repeat due to less than 0.5g of tissue being collected.

18 Refer to Section 9.3. To be drawn the day of the tissue biopsy.

19 Refer to Section 9.4 PK [pharmacokinetics] and 14.2 PD pharmacodynamics)[proteasome inhibition]Cycle 1 only

20 As per BMT services

21 BM biopsy and aspiration to be completed within 28 days prior to enrollment

22 If biopsy is completed at an outside facility or Network Site, a tissue block or slides will be submitted to the Roswell Park Pathology Department for central pathology review. In addition immunohistochemistry (IHC) for CD20, Ki67, CD10, Bcl-2, Bcl-6, and MUM-1 staining will be performed. Cell of origin will be determined according to the Han's algorithm as previously described. Samples may only be shipped to Roswell Park Monday-Thursday.

23 For patients within the first 3 years of follow up, CT scans will be ordered every other visit. Patients on annual follow up will receive a CT scan at every study visit until the end of year 5, then CT scans will be ordered as clinically indicated

24 PK/PD samples will be collected in Phase 1 only

9.2 Pathology

If a patient was confirmed at Roswell Park or an outside institute for CD20-positive DLBCL, then the diagnosis will be confirmed by centrally reviewing the available pathology material or the outside material utilized for this purpose. If confirmation is needed on outside material, a representative block or 15 unstained 4 μm sections on 15 charged slides will be requested for this purpose.

Immunohistochemistry (IHC) will be utilized, if not already performed, to confirm the diagnosis of DLBCL and subtype it as Germinal Center (GC) or non-GC-like based on variable expression of CD10, BCL6, and MUM1 (based on Hans algorithm). Additional IHC markers that could be used, most of which are part of routine standard of care work up, include but not restricted to CD20, BCL1 (to exclude mantle cell lymphoma), BCL2, and ki-67.

A fresh lymph node/tumor biopsy needs to be repeated only for diagnostic purposes or if the patient has received additional chemotherapy after their previous biopsy. Fresh tissue or paraffin imbedded/unstained slides (N=15) (For Network Institutions) will be submitted to the department of pathology for standard and research protocol work-up. The tissue will be divided into standard of care diagnostic tissue (for H-E and IHC work-up, among others if needed such as flow cytometry immunophenotypic analyses and cytogenetics) and fresh tissue sent to Roswell Park Department of Medicine Lymphoma/Myeloma Laboratory (if sufficient tissue). Contact Cory Mavis at 716-868-5333 or 716-845-3464 for additional information.

Outside material should be sent to Dr. Vishala Neppalli, Pathology Department, Roswell Park Comprehensive Cancer Center, Elm and Carlton Streets, Buffalo NY 14263.

9.3 Immune Biomarker Blood Sample Collection

If the patient is undergoing a fresh lymph node/tumor biopsy, the following samples will be collected. Samples of blood will be collected using (5) 10 mL green-top heparinized tubes (1 tube for phenotype in flow cytometry, and 4 tubes for CMC/ADCC), (1) gold-tube (for CMC), and (1) 10 mL lavender-top tube (for genotypic analysis). Samples will be collected on the same day as the lymph node biopsy. Network Sites will collect immune biomarker samples on day of tumor biopsy if it is after the informed consent. Fresh tissue will not be collected from Network Sites.

One green top tube will be sent to the flow cytometry department and the remainder of the samples will be sent to Roswell Park Department of Medicine Lymphoma/Myeloma Laboratory for processing: Tube station #621 in Roswell Park Cancer Cell Center.

For additional information regarding the handling of samples please contact Cory Mavis at 716-868-5333 or 716-845-3464.

Refer to **Section 14.1** for analysis details.

9.4 Pharmacokinetic Blood Sample Collection and Processing (Phase 1 Only)

Whole blood samples for the pharmacokinetic analysis of carfilzomib levels will be collected via venipuncture using 4 mL green-top heparinized tubes.

PK samples will be collected pre-dose, just prior to the end of the infusion; and at 15 minutes, 30 minutes, 1, 2, 4, 6 hours post infusion on Cycle 1, Day 1, then at 24 hours post Cycle 1 infusion on Cycle 1, Day 2 (prior to Day 2 infusion).

PK samples will be sent to procurement lab. PD samples will be sent to Roswell Park Department of Medicine Lymphoma/Myeloma Laboratory for processing: Tube station #621 in Roswell Park Cancer Cell Center.

Plasma will be separated from the whole blood within 30 minutes following the extraction. The screw cap polypropylene cryogenic tube will be labeled with the subject's initials, subject's study number, clinical study number, protocol time point, dose, and protocol day. The samples will be frozen at -70°C or below until analyzed. After hours, samples will be processed in Lab Medicine.

The concentrations of carfilzomib in plasma will be determined using a validated LC/MS/MS method. Drug extraction will consist of protein precipitation with acetonitrile. The analytes will be separated on a C18 column with mobile phase consisting of acetonitrile, water and 0.1% formic acid. The method will be cross-validated before applying to the specimens for quantification. Quality assurance will be maintained by injecting quality control samples each time the samples are assayed.

Samples will be analyzed in Roswell Park Bioanalytics, Metabolomics & Pharmacokinetics Core Facility.

Roswell Park
Bioanalytics, Metabolomics & Pharmacokinetics Core Facility
Center for Genetics and Pharmacology, Room L1-140
Refer to Study Number– I 240813
Elm & Carlton Streets
Buffalo, New York 14263
PKPDCore@RoswellPark.org

For additional information regarding the handling of pharmacokinetic samples please contact Roswell Park's Bioanalytics, Metabolomics & Pharmacokinetics Core Facility laboratory at 716-845-3303 (Tel) or 716-845-1579 (Fax).

Refer to **Section 14.2** for analysis details.

Note: All investigator or analyzing research laboratories housing research samples need to maintain current Temperature Logs and study-specific Sample Tracking and Shipping Logs. The Principal Investigator/Laboratory Manager must ensure that the stated lab(s) have a process in place to document the receipt/processing/storage/ shipping of study-related samples/specimens. This is required for both observational and interventional clinical studies collecting clinical samples.

10 EFFICACY EVALUATIONS

10.1 Objectives

One of the secondary objectives of this clinical study is to evaluate the overall response rate and complete response rate to C-R-ICE in rel/ref DLBCL. It is important to evaluate response to therapy using standardized response criteria utilized in previous clinical studies in DLBCL patients so direct comparisons can be performed with historical controls (i.e., R-ICE). For this reason response to therapy will be evaluated using the response criteria reported by Cheson published in 2007 which incorporate whole body PET scan in addition to CT scanning.

10.2 Imaging, Laboratory and Pathological Studies

Imaging, laboratory and pathological studies will be conducted for staging and response evaluation at baseline, between Cycle 2 Day 15 and Cycle 2 Day 28, and at the end of study evaluation following Cycle 3. Response to treatment will be evaluated according to the International Working Group Response criteria as reported by the revised Cheson criteria (**Section 10.3**).

10.3 Modified Cheson Criteria

10.3.1 Complete Response (CR)

- Complete disappearance of all detectable clinical evidence of disease and disease-related symptoms if present before therapy.
- Typically FDG-avid lymphoma: in patients with PET scan positive before therapy, a post-treatment residual mass of any size is permitted as long as it is PET negative.
- Variably FDG-avid lymphomas/FDG avidity unknown: in patients with a negative pretreatment PET scan, all lymph nodes and nodal masses must have regressed on CT 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 cm to 1.5 cm in their long axis and more than 1.0 cm in their short axis before treatment must have decreased to 1.0 cm in their short axis after treatment.
- The spleen and/or liver, if considered enlarged before therapy on the basis of a physical examination or CT scan, should not be palpable on physical examination and should be considered normal size by imaging studies, and nodules related to lymphoma should disappear. However, determination of splenic involvement is not always reliable because a spleen considered normal in size may still contain lymphoma, whereas an enlarged spleen may reflect variations in anatomy, blood volume, the use of hematopoietic growth factors, or causes other than lymphoma.
- If the bone marrow was involved by lymphoma before treatment the infiltrate must have cleared on repeat bone marrow biopsy.

- The bone marrow biopsy sample on which this determination is made must be adequate (with a goal of 20 mm unilateral core). If the sample is indeterminate by morphology, it should be negative by immunohistochemistry. A sample that is negative by immunohistochemistry but that demonstrates a small population of clonal lymphocytes by flow cytometry will be considered a CR until data become available demonstrating a clear difference in patient outcome.

10.3.2 Partial Response (PR)

- A decrease of ≥ 50% in the in the sum of the products of their greatest transverse diameters (SPD) of the 6 largest dominant nodes or nodal masses. These nodes or masses should be selected according to the following features: a) they should be clearly measurable in at least two perpendicular measurements; b) they should be from as disparate regions of the body as possible; and c) they should include mediastinal and retroperitoneal areas of disease whenever these sites are involved.
- No increase in the size of other nodes, liver, or spleen.
- Spleen and hepatic nodules must regress by at least 50% in SPD.
- With the exception of splenic and hepatic nodules, involvement of other organs is considered assessable and not measurable disease.
- Bone marrow assessment is irrelevant for determination of a PR because it is assessable and not measurable disease; however, if positive the cell type should be specified in the report (e.g., large-cell lymphoma).
- Patients who achieve a CR by the above criteria, but who have persistent morphologic bone marrow involvement will be considered partial responders.
- When the bone marrow was involved before therapy and a clinical CR was achieved, but with no bone marrow assessment after treatment, patients should be considered partial responders.
- No new sites of disease should be observed.
- Typically FDG-avid lymphoma: for patients with no pretreatment PET scan or if the PET scan was positive before therapy, the post-treatment PET should be positive in at least one previously involved site.
- Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan, or if a pretreatment PET scan was negative, CT criteria should be used.

10.3.3 Stable Disease (SD)

- A patient is considered to have SD when he or she fails to attain the criteria needed for a CR or PR, but does not fulfill those for progressive disease (see Relapsed Disease [after CR]/Progressive Disease [after PR, SD]).

- Typically FGD-avid lymphomas: the PET should be positive at prior sites of disease with no new areas of involvement on the post-treatment CT or PET.
- Variably FDG-avid lymphomas/FDG-avidity unknown: for patients without a pretreatment PET scan or if the pretreatment PET was negative, there must be no change in the size of the previous lesions on the post-treatment CT scan.

10.3.4 Progression (PD) or Relapse

- Lymph nodes should be considered abnormal if the long axis in more than 1.5 cm regardless of the short axis. If a lymph node has a long axis of 1.1 cm to 1.5 cm, it should only be considered abnormal if its short axis is more than 1 cm. Lymph nodes 1 cm x 1 cm will not be considered as abnormal for relapse or progressive disease.
- Appearance of any new lesion more than 1.5 cm in any axis during or at the end of therapy, even if other lesions are decreasing in size. Increased FDG uptake in a previously unaffected site should only be considered relapsed or progressive disease after confirmation with other modalities. In patients with no prior history of pulmonary lymphoma, new lung nodules identified by CT are mostly benign. Thus, a therapeutic decision should not be made solely on the basis of the PET without histologic confirmation.
- At least a 50% increase from nadir in the SPD of any previously involved nodes, or in a single involved node, or the size of other lesions (e.g., splenic or hepatic nodules). To be considered progressive disease, a lymph node with a diameter of the short axis of less than 1 cm must increase by 50% and to a size of 1.5 cm or more than 1.5 cm in the long axis.
- At least a 50% increase in the longest diameter of any single previously identified node more than 1 cm in its short axis.
- Lesions should be PET positive if observed in a typical FDG avid lymphoma or the lesion was PET positive before therapy unless the lesion is too small to be detected with current PET systems (1.5 cm in its long axis by CT).

10.3.5 Duration of Overall Response and/or Progression-Free Survival (in Non-Responders)

- The duration of overall response is measured from the time measurement criteria are met for CR/PR (whichever is first recorded) until the first date that recurrent or progressive disease is objectively documented (taking as reference for progressive disease the smallest measurements recorded since the treatment started).
- The duration of overall complete response is measured from the time measurement criteria are first met for CR until the first date that recurrent disease is objectively documented.
- Duration of Stable Disease

- Stable disease is measured from the start of the treatment until the criteria for progression are met, taking as reference the smallest measurements recorded since the treatment started.

10.4 Duration of Overall Survival

Duration of Overall Survival is measured from the start of treatment until death for any reason (cause of death will be documented whenever possible). Patients that progress at any time will be followed for survival only, on a yearly basis either by review of medical records or a phone call.

11 SAFETY EVALUATION

11.1 Adverse Events

11.1.1 Definition

An adverse event or adverse experience (AE) is any untoward medical occurrence associated with the use of a drug in humans, whether or not considered drug related. Therefore, an AE can be ANY unfavorable and unintended sign (including an abnormal laboratory finding), symptom, or disease temporally associated with the use of a medicinal (investigational) product, whether or not considered related to the medicinal (investigational) product (attribution of 'unrelated', 'unlikely', 'possible', 'probable', or 'definite').

An AE is considered "unexpected" if it is not listed in the investigator brochure or is not listed at the specificity or severity that has been observed; or if an investigator brochure is not required or available, is not consistent with the risk information described in the general investigational plan in other study-related documents.

11.1.1.1 Diagnosis Versus Signs and Symptoms

If known, a diagnosis should be recorded on the CRF rather than individual signs and symptoms (e.g., record only liver failure or hepatitis rather than jaundice, asterixis, and elevated transaminases). However, if a constellation of signs and/or symptoms cannot be clinically characterized as a single diagnosis or syndrome at the time of reporting, each individual event should be recorded as an AE or SAE on the CRF. If a diagnosis is subsequently established, it should be reported as follow-up information.

11.1.1.2 Adverse Events Occurring Secondary to Other Events

In general, AEs occurring secondary to other events (e.g., cascade events or clinical sequelae) should be identified by their primary cause. For example, if severe diarrhea is known to have resulted in dehydration, it is sufficient to record only diarrhea as an AE or SAE on the CRF.

However, clinically significant AEs occurring secondary to an initiating event that are separated in time should be recorded as independent events on the CRF. For example, if a severe gastrointestinal hemorrhage leads to renal failure, both events should be recorded separately on the CRF.

11.1.1.3 Abnormal Laboratory Values

Only clinically significant laboratory abnormalities that require active management will be recorded as AEs or SAEs on the CRF (e.g., abnormalities that require study drug dose modification, discontinuation of study treatment, more frequent follow-up assessments, further diagnostic investigation, etc.).

If the clinically significant laboratory abnormality is a sign of a disease or syndrome (e.g., alkaline phosphatase and bilirubin 5 x the upper limit of normal associated with cholecystitis), only the diagnosis (e.g., cholecystitis) needs to be recorded on the Adverse Event CRF.

If the clinically significant laboratory abnormality is not a sign of a disease or syndrome, the abnormality itself should be recorded as an AE or SAE on the CRF. If the laboratory abnormality can be characterized by a precise clinical term, the clinical term should be recorded as the AE or SAE. For example, an elevated serum potassium level of 7 mEq/L should be recorded as "hyperkalemia"

Observations of the same clinically significant laboratory abnormality from visit to visit should not be repeatedly recorded as AEs or SAEs on the CRF, unless their severity, seriousness, or etiology changes.

11.1.1.4 Preexisting Medical Conditions (Baseline Signs and Symptoms)

A preexisting medical condition should be recorded as an AE or SAE only if the frequency, severity, or character of the condition worsens during the study. When recording such events on an Adverse Event CRF, it is important to convey the concept that the preexisting condition has changed by including applicable descriptors (e.g., "more frequent headaches").

11.1.2 Grading and Relationship to Drug

The descriptions and grading scales found in the CTEP Version 4 of the NCI Common Terminology Criteria for Adverse Events (CTCAE) will be utilized for AE reporting. CTEP Version 4 of the CTCAE is identified and located at: http://ctep.cancer.gov/protocolDevelopment/electronic_applications/ctc.htm. AEs not covered by specific criteria should be reported with common medical terminology and graded according to definitions provided in the CTCAE Version 4.

The relationship of event to study drug will be documented by the Investigator as follows:

- **Unrelated:** The event is clearly related to other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs administered to the patient.
- **Unlikely:** The event is doubtfully related to investigational agent(s). The event was most likely related to other factors such as the patient's clinical state, other therapeutic interventions, or concomitant drugs.
- **Possible:** The event follows a reasonable temporal sequence from the time of drug administration, but could have been produced by other factors such as the patient's clinical state, other therapeutic interventions or concomitant drugs.

- **Probable:** The event follows a reasonable temporal sequence from the time of drug administration, and follows a known response pattern to the study drug. The event cannot be reasonably explained by other factors such as the patient's clinical state, therapeutic interventions or concomitant drugs.
- **Definite:** The event follows a reasonable temporal sequence from the time of drug administration, follows a known response pattern to the study drug, cannot be reasonably explained by other factors such as the patient's condition, therapeutic interventions or concomitant drugs; AND occurs immediately following study drug administration, improves upon stopping the drug, or reappears on re-exposure.

11.1.3 Reporting Adverse Events

Table 4. Guidelines for Routine Adverse Event Reporting for Phase I Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated	X	X	X	X
Unlikely	X	X	X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

Table 5. Guidelines for Routine Adverse Event Reporting for Phase Ib Studies (Regardless of Expectedness)

Attribution	Grade 1	Grade 2	Grade 3	Grade 4
Unrelated			X	X
Unlikely			X	X
Possible	X	X	X	X
Probable	X	X	X	X
Definite	X	X	X	X

All new routine AEs occurring between the start date of intervention until 30 days after the last intervention or until the event has resolved, stabilized, death, or a new treatment is started, whichever comes first, will be reported.

11.2 Serious Adverse Events

11.2.1 Definition

A serious adverse event (SAE) is any adverse event (experience) that in the opinion of either the investigator or sponsor results in **ANY** of the following:

- Death.

- A life-threatening adverse event (experience). Any AE that places a patient or patient, in the view of the Investigator or sponsor, at immediate risk of death from the reaction as it occurred. It does NOT include an AE that, had it occurred in a more severe form, might have caused death.
- Inpatient hospitalization or prolongation of existing hospitalization (for > 24 hours).
- A persistent or significant incapacity or substantial disruption of the ability to conduct normal life functions.
- A congenital anomaly or birth defect.
- Important Medical Event (IME) that, based upon medical judgment, may jeopardize the patient or patient and may require medical or surgical intervention to prevent one of the outcomes listed above.

11.2.2 Reporting Serious Adverse Events

All new SAEs occurring from the date the patient signs the study consent until 30 days after the last intervention or a new treatment is started, whichever comes first, will be reported. SAE's occurring after this time that the investigator determines to be possibly, probably or definitely related to the study intervention, should be reported.

The Roswell Park SAE Source Form is to be completed with all available information, including a brief narrative describing the SAE and any other relevant information. The site Investigator or designated research personnel will report all SAEs, whether related or unrelated, to the investigational agent(s) to the IRB in accordance with their local institutional guidelines.

SAE's identified as an Unanticipated Problem by the Investigator must be reported. Please refer to Section 11.5 for details on reporting Unanticipated Problems.

Amgen should receive copies of all safety reports submitted to the FDA, or any regulatory agency, IRB or IEC, within 24 hours of such submission. Such notifications should be submitted as MedWatch reports using the attached SAE fax cover letter and faxed to 1-888-814-8653. Note the fax cover must contain both Amgen and Onyx study numbers, as reflected in the attached form (Appendix D).

11.3 Investigator Reporting: Notifying the Study Sponsor

Notify the study sponsor: SAEs should be reported to Amgen by fax to: **+1 (888) 814-8653**.

11.4 Follow-Up for Serious Adverse Events

All SAEs will be followed until resolution, stabilization, death, or the start of new treatment. New information will be reported when it is received.

11.5 Unanticipated Problems

11.5.1 Definition

An Unanticipated Problem (UP) is any incident, experience, or outcome that meets all of the following criteria:

- Unexpected (in terms of nature, severity, or frequency) given:
  a) The research procedures that are described in the study-related documents, including study deviations, as well as issues related to compromise of patient privacy or confidentiality of data.
  b) The characteristics of the patient population being studied.
- Related or possibly related to participation in the research (possibly related means there is a reasonable possibility that the incident, experience, or outcome may have been caused by the procedures involved in the research).
- Suggests that the research places patients or others at a greater risk of harm (including physical, psychological, economic, or social harm) than was previously known or recognized and if in relation to an AE is also deemed Serious per **Section 11.2**.

11.5.2 Reporting Unanticipated Problems

The Unanticipated Problem Form will be submitted to CRS Compliance Office within 1 business day of becoming aware of the Unanticipated Problem.

When becoming aware of new information about the Unanticipated Problem, submit this updated information to CRS Compliance with an updated Unanticipated Problem Form. The site Investigator or designated research personnel will report all unanticipated problems, whether related or unrelated to the investigational agent(s) to the **IRB in accordance with their local institutional guidelines**.

11.6 FDA Reporting

When Roswell Park is the IND holder the following describes the FDA reporting requirements by timeline for AEs and new safety findings that meet the criteria outlined below:

Within 7 Calendar Days

Any adverse event that meets **ALL** the following criteria:

Related or possibly related to the use of the study drug;

Unexpected; and Fatal or life-threatening.

Within 15 Calendar Days

Any adverse event that meets **ALL** the following criteria:

Related or possibly related to the use of the study drug;

Unexpected; and Serious but not fatal or life-threatening.

Or meets **ANY** of the following criteria:

A previous adverse event that is not initially deemed reportable but is later found to fit the criteria for reporting (report within 15 days from when event was deemed reportable).

Any findings from other studies, including epidemiological studies, pooled analysis of multiple studies, or other clinical studies conducted with the study drug that suggest a significant risk in humans exposed to the drug.

Any findings from animal or in vitro testing that suggest a significant risk for human patients including reports of mutagenicity, teratogenicity, or carcinogenicity or reports of significant organ toxicity at or near the expected human exposure.

Any clinically important increase in the rate of occurrence of a serious, related or possibly related adverse event over that listed in the protocol or investigator brochure.

Sponsors are also required to identify in IND safety reports, all previous reports concerning similar adverse events and to analyze the significance of the current event in the light of the previous reports.

Reporting Process

The principal investigator or designee will complete and submit a FDA Form 3500A MedWatch for any event that meets the above criteria. Forms will be submitted to the CRS Compliance Office via email to: CRSCompliance@RoswellPark.org.

12 DATA AND SAFETY MONITORING

Phase I studies will be reviewed at the scheduled Roswell Park Phase I meetings and the minutes are forwarded to the IRB for review.

The Roswell Park Data and Safety Monitoring Board will assess the progress of the study, the safety data, and critical efficacy endpoints. The DSMB will review the study annually and will make recommendations that include but not limited to; (a) continuation of the study, (b) modifications to the design (c) or termination of the study.

13 STATISTICAL METHODOLOGY

This is single-center, open-label, prospective Phase I/Ib clinical trial evaluating the safety, efficacy, and pharmacokinetics/pharmacodynamics of carfilzomib and standard R-ICE combination therapy in adult patients with relapsed/refractory diffuse large B-cell lymphoma. The statistical design is based on a dose-finding algorithm to determine the maximal tolerated dose (MTD) of the study regimen and an assessment of the efficacy of the treatment for patients as compared to an external standard, i.e., R-ICE historical control. All enrolled patients will receive the study drug and initiation of the treatment should take place as soon as possible following study enrollment. A maximum total of up to 21 evaluable patients will be enrolled in the Phase I of this study, and a total of 9 evaluable patients will be enrolled in the Phase Ib portion of the study. An evaluable subject is defined as a subject who meets eligibility requirements and who does not discontinue prior to end of Cycle 1 for reasons other than DLTs for Phase I or who completes at least 1 cycle of planned therapy for Phase Ib. If a subject signs

an Informed Consent and does not receive treatment, the subject will not be classified as evaluable and will be replaced. Unevaluable patients will be replaced. Analyses will be performed with a focus on estimation of specific clinically important parameters for use in the planning of larger subsequent comparative trials designed to fully assess efficacy and safety.

13.1 Sample Size Determination

13.1.1 Phase I

The actual sample size for the Phase I population depends on the unknown dose-toxicity profile of the treatment and the sample size is 21.

13.1.2 Phase Ib

The sample size calculation for the Phase Ib population is based on testing the hypotheses concerning the proportion of the population who experience an objective response (i.e., CR or PR) described above. This design requires a total of up to 30 patients in order to achieve approximately 80.1% power to detect differences of 20 percentage points (50% versus 70%).

Based on the excellent response observed in the patients treated in the Phase I portion (ORR 90%) and budget changes, the sample size for the Phase Ib portion was reduced. The phase Ib portion will include 9 additional patients. The efficacy analysis will include evaluable patients in the Phase I and Phase Ib portions combined (sample size up to 45). The sample size calculation for the efficacy analyses is based on testing the hypotheses concerning the proportion of the population who experience an objective response (i.e., CR or PR) described above. This design requires a total of up to 30 patients in order to achieve approximately 80.1% power to detect differences of 20 percentage points (50% versus 70%).

13.2 Missing Data

The amount and nature of missing data will be characterized and no method of imputation will be used for missing data. A summary of missing data will be provided according to the number of subjects, the time points where the data are missing, and clinical center. For each clinical center, the number and percent of subjects with no missing data will be presented in tabular form.

13.3 Descriptive Analyses

Measured outcome variables will be summarized overall and by relevant demographic and baseline variables. Descriptive statistics such as frequencies and relative frequencies will be computed for all categorical variables. Numeric variables will be summarized using simple descriptive statistics such as the mean, standard deviation and range. A variety of graphical techniques will also be used to display data, ex. histograms, boxplots, scatterplots, etc.

13.4 Phase I

The primary objective of the Phase I part of this study is to determine the MTD of the proposed regimen defined as the dose of carfilzomib added to standard R-ICE chemotherapy which, if exceeded, would put the patient at an undesirable risk of medically unacceptable, DLTs experienced during treatment with C-R-ICE within the treated patient population. The definition of a DLT is in **Section 7.4.1**.

The basic design of the Phase I trial will utilize a standard 3+3 dose-finding scheme which is a special case of the A + B design described by Lin and Shih (2001). Rationale behind the design is nested in the assumption that both the probabilities of toxicity and efficacious response are continuous monotonic non-decreasing functions of the dose.

13.5 Phase Ib (expansion cohort)

The objective of the Phase Ib component is examination of the efficacy and toxicity of the addition of carfilzomib to R-ICE at the MTD dose.

The primary objective of the Phase Ib component is to assess the efficacy of the study treatment arm as compared to historical control. The primary efficacy endpoint will be best overall response rate (PR+CR) within following 3 cycles of C-R-ICE therapy (as demonstrated by whole body PET-CT and dedicated CT scans following Cycle 3 of therapy).

The efficacy analysis will include patients treated in the Phase I and Phase Ib part of the study. This change in the protocol was decided based on the excellent ORR observed in the first 15 patients treated in the Phase part of the study and changes in the protocol budget.

13.5.1 Historical Control

Patients in this population defined by the eligibility criteria receive standard-of-care which consist of blood product support as needed and growth factor support with each cycle. Based on provided historical information, the overall response rate in this patient population receiving standard-of-care is 50%. It is anticipated that treatment with carfilzomib added to standard R-ICE will increase the overall response rate beyond the clinically significant rate of 70%.

13.5.2 Phase Ib Decision Rules

Let π represent the proportion of the evaluable population of interest who experience an overall response within 3 weeks after completion of therapy (i.e., 3 cycles) at the carfilzomib MTD. If the response rate of less than 50% is considered unacceptable. A response rate of more than 70% is considered promising and evidence of such will deem the treatment worthy of further study. The null and alternative hypotheses corresponding to the design are

$$H_0 : \pi \leq 50\%,$$
$$H_a : \pi > 50\%.$$

A total of up to 30 evaluable patients will be accrued in the Phase I and Phase Ib portions of the study. The evaluable population is defined as patients who meet eligibility requirements and complete at least 1 cycle of therapy. Unevaluable patients will be replaced.

With this re-design, the probability is 4.8% of falsely concluding that the proportion of those who experience an overall response exceeds 50% and 80.1% is the probability of correctly concluding efficacy when the overall response rate is 70%.

In the event that the actual first stage sample size is not as planned, the adjustment of the first-stage stopping rule will be based on the approximate minimax rule proposed in Green and Dahlberg (1992): Stop the trial if the number of responses is α or less, where *a* is the largest value for which power properties would be maintained given the attained first-stage sample size, assuming the planned final sample size is achieved and one applies the planned final boundary. If the planned second stage sample size is not attained, the bound will be adjusted so to preserve Type I error.

13.5.3 Secondary Analyses

Both efficacy and toxicity rates will be estimated using simple relative frequencies. The corresponding 95% confidence intervals for the estimated probabilities will be computed using the method proposed in Clopper and Pearson (1934). The relationship between binary outcomes and collected demographic and baseline variables will be statistically assessed using logistic regression in an exploratory fashion. Maximum likelihood estimation will be utilized in the model fitting procedures as implemented by SAS PROC LOGISTIC. Wald tests of the model effects will be performed to assess statistical significance; see SAS documentation for details. See Hosmer and Lemeshow (2000) for a discussion of such techniques. Alternative parametric models will be considered if model fit is found to be inadequate.

The estimated distributions of overall and progression free survival will be obtained using the Kaplan-Meier method. Estimates of quantities such as median survival will be obtained. Corresponding confidence intervals using the methodology of Brookmeyer and Crowley (1982) will be computed. It is assumed a priori that any drop out times will be non-informative in terms of the censoring mechanism. Groups defined by levels of categorical or dichotomized numeric demographic/baseline variables will be compared in regards to time-to-event distributions using the log-rank test. Cox proportional hazards model regression will be utilized for multivariate analyses.

All tests will be two-sided and tested at a 0.05 nominal significance level. Standard diagnostic plots will be used to assess model fit and transformations of variables may be considered in order to meet statistical assumptions.

The analysis will be performed using the SAS® statistical software system.

14 CORRELATIVE STUDIES

14.1 Immune Biomarker Analysis

14.1.1 Blood

- Phenotypic Studies by direct flow cytometric analysis of neutrophils, NK and monocytes for the expression of CD11b/CD18; CD32, CD33, CD62, CD64, CD69, and

CD56 vFunctional Assays in vitro. (1) 10 ml green top – same day as lymph node biopsy

- Antibody-dependent cellular cytotoxicity (ADCC) assays: The capacity to lyse autologous tumor cells in the presence of RITUXAN by peripheral blood mononuclear cells of each patient will be studied by standardized assays, including appropriate controls. (4) 10 ml green tops – same day as lymph node biopsy
- Complement mediated cytotoxicity (CMC) assays: The capacity to lyse autologous tumor cells in the presence of RITUXAN by serum from each patient will be studied by standardized assays, including appropriate controls. (1) gold top – same day as lymph node biopsy
- Genotypic Analysis: Polymorphisms in the IgG Fc receptor FcγIIIa gene will be performed using a nested PCR followed by allele-specific restriction enzyme digestion performed in Roswell Park Department of Medicine Lymphoma/Myeloma Laboratory.(1) 10 ml lavender top – same day as lymph node biopsy
- Assay for proteasome inhibition of patient-derived primary DBLCL cells and PBMCs will be performed using an enzymatic assay for chymotrypsin-like activity. (1) 10 ml green top with each designated time point in Cycle 1 (see section 14.2)

14.1.2 Fresh Tissue (Phase I part only)

- Autologous neoplastic B-cells derived from fresh tissue samples (lymph node biopsy specimen or organ specimen) will be exposed ex vivo to carfilzomib versus bortezomib +/- rituxan (including appropriate controls). Response to therapy will be measured. Additionally, genetic profiling (microarray), to determine activated B-cell (ABC) versus germinal B-cell (GBC) phenotype and protein profiling (protein arrays), in particular, changes in Bcl-2 family protein profile and/or inactivation of NF-κB signaling after ex vivo exposure of NHL cells to carfilzomib (compared to bortezomib), will be performed to examine potentially prognostic factors for response to C-R-ICE.

14.2 PK/PD Model Development

A pharmacologically-driven PK/PD model will be developed to elucidate the temporal relationship between carfilzomib concentrations and the time course of proteasome inhibition, along with temporal relationships with thrombocytopenia and neutropenia. This model will allow assessment of the dose and schedule at which carfilzomib facilitates maximal proteasome inhibition and thus lead to an increase response rate, while modulating the degree of severe toxicities. In addition, the effect of concomitant administration of rituximab, ifosfamide, carboplatin, and etoposide will be incorporated into the model to assess their potential effects on efficacy and/or toxicity.

First, a variety of compartmental population PK structural models will be evaluated to characterize the plasma concentration versus time profile following administration of carfilzomib using a nonlinear mixed effects modeling approach NONMEM®. The physiologic pharmacokinetic models explored will be described by the estimation of mean structural model

parameters (e.g. plasma volumes of distribution and clearances), the magnitude of inter-animal variability (IIV) in these parameters and the magnitude of residual variability (RV). For each model, the fit will be assessed by examination of several diagnostics. For comparisons of hierarchical models, the change in the minimum value of the objective function (MVOF), a statistic that is proportional to minus twice the log likelihood of the data, will be examined.

Using a sequential approach, a population PK/PD structural model will be developed for carfilzomib based on degree of proteasome inhibition in relation to efficacy and toxicity endpoints in the proposed study using NONMEM. This PK/PD model will describe the potential relationship between carfilzomib exposure in relation to proteasome inhibition to the time course of thrombocytopenia and neutropenia as indicators of pharmacodynamic response. The relationship between carfilzomib concentrations and these pharmacodynamic endpoints will be initially explored graphically and various structural models will be applied to the data and evaluated. Similar to the PK model development, criteria such as the precision of parameter estimates, magnitude of residual variability, and goodness-of-fit will be utilized for PK/PD model selection. The PK/PD model could be expanded to include attributes that are indicators of pharmacodynamic response from the concomitant medications of rituximab, ifosfamide, cisplatin, and etoposide which will modulate the overall response rate.

Assay for Proteasome Inhibition. This measures (but does not distinguish from) the activity of beta5 (constitutive proteasome) and LMP7 (immunoproteasome). Analysis of biomarkers that predict for response following treatment with proteasome inhibitors will also be performed on PBMC and whole blood plasma samples ((1) 10 ml green top tube with each blood draw) taken at time points below in Cycle 1 only: Day 1 of Cycle 1, pre-dose and 1 hr post end of infusion; Day 2 of Cycle 1, pre-dose and 1 hr post end of infusion and Day 3, Cycle 1, prior to rituximab administration..

Samples will be sent to Roswell Park Department of Medicine Lymphoma/Myeloma Laboratory for processing. Tube station #621 in Roswell Park Cancer Cell Center.

For additional information regarding the handling of samples please contact Cory Mavis at 716-868-5333 or 716-845-3464.

Furthermore, the final PK/PD model will be used to conduct a clinical trial simulation to predict pharmacokinetic outcomes of various dosing regimens (94, 95) to guide dosing and scheduling of C-R-ICE in relation to safety and efficacy endpoints. The simulation results will allow prediction of the exposure-response relationships for different doses and schedules of carfilzomib to achieve the desired responses.

15 ETHICAL AND REGULATORY STANDARDS

15.1 Ethical Principles

This study will not be initiated until the protocol and informed consent document(s) have been reviewed and approved by a properly constituted Institutional Review Board (IRB) or Independent Ethics Committee (IEC). Each subject (or legal guardian) shall read, understand, and sign an instrument of informed consent prior to performance of any study-specific

procedure. It is the responsibility of the investigator to ensure that the subject is made aware of the investigational nature of the treatment and that informed consent is given.

The Investigator is responsible for the retention of the subject log and subject records; although personal information may be reviewed by authorized persons, that information will be treated as strictly confidential and will not be made publicly available. The investigator is also responsible for obtaining subject authorization to access medical records and other applicable study specific information according to Health Insurance Portability and Accountability Act regulations (where applicable).

This study will be conducted in compliance with all applicable laws and regulations of the state and/or country and institution where the subject is treated, in accordance with the Declaration of Helsinki, Good Clinical Practice, and according to the guidelines in this protocol, including attached appendices.

15.2 Informed Consent

The Investigator is responsible for obtaining written consent from each subject or the subject's legally authorized representative in accordance with ICH-GCP guidelines using the approved informed consent form, before any study specific procedures (including screening procedures) are performed. The informed consent form acknowledges all information that must be given to the subject according to ICH-GCP, including the purpose and nature of the study, the expected efficacy and possible side effects of the treatment(s), and specifying that refusal to participate will not influence further options for therapy. Any additional information that is applicable to the study must also be included. Additional national or institutionally mandated requirements for informed consent must also be adhered to. The subject should also be made aware that by signing the consent form, processing of sensitive clinical trial data and transfer to other countries for further processing is allowed.

The Investigator shall provide a copy of the signed consent form to the subject and the signed original shall be maintained in the Investigator File. A copy of the signed consent form must be filed in the subject file. At any stage, the subject may withdraw from the study and such a decision will not affect any further treatment options.

16 STUDY RESPONSIBILITIES

16.1 Data Collection

Data entry into the database is to be completed in a timely fashion (approximately within 28 days) after the subject’s clinic visit. If an AE is considered serious it is captured on both the Adverse Event page and the Serious Adverse Event Form, which is handled in an expedited fashion.

Data management activities will be performed using EXPeRT. EXPeRT is a suite of software tools that enables the collection, cleaning and viewing of clinical trial data. CRS data management will design the study-specific database and facilitate its development by the EXPeRT Information Technology team. Once the database design is approved by the Investigator, Statistician, and Clinical Research Coordinator, the database will be put into

production and data entry can begin. Data can be entered and changed only by those with the rights to do so into the eCRFs. EXPeRT is compliant with all relevant technical aspects of relevant GCP guidelines.

- The system can generate accurate copies of stored data and audit trail information in human readable form.
- System access is limited to authorized individuals through the controlled assignment of unique ID and password combinations.
- The system is designed to periodically force users to change their passwords and verifies that user ID and password combinations remain unique.
- The system automatically generates a permanent time-stamped audit trail of all user interactions.

When data entry is complete, data management will review the data and will query any missing, incomplete, or invalid data points for resolution by the Clinical Research Coordinator and Investigator. Once all queries have been resolved, the data can be released to the statistician for analysis.

16.2 Maintenance of Study Documents

Essential documents should be retained per Roswell Park policy for 6 years from the study termination date. These documents could be retained for a longer period, however, if required by the applicable local regulatory requirements or by an agreement with Roswell Park. It is the responsibility of Roswell Park to inform the Investigator/institution as to when these documents no longer need to be retained. If, for any reason, the Investigator desires to no longer maintain the study records, they may be transferred to another institution, another investigator, or to Roswell Park upon written agreement between the Investigator and Roswell Park.

17 ADMINISTRATIVE RULES

17.1 Revisions to the Protocol

Roswell Park may make such changes to the protocol as it deems necessary for safety reasons or as may be required by the U.S. FDA or other regulatory agencies. Revisions will be submitted to the IRB/ERC for written approval before implementation.

17.2 Termination of the Study

It is agreed that, for reasonable cause, either the Investigators or the Sponsor, Roswell Park may terminate this study, provided a written notice is submitted within the time period provided for in the Clinical Trial Agreement. In addition, Roswell Park may terminate the study at any time upon immediate notice if it believes termination is necessary for the safety of subjects enrolled in the study.

17.3 Confidentiality

Any data, specimens, forms, reports, video recordings, and other records that leave the site will be identified only by a participant identification number (Participant ID, PID) to maintain confidentiality. All records will be kept in a limited access environment. All computer entry and networking programs will be done using PIDs only. Information will not be released without written authorization of the participant.

18 APPENDICES

Appendix A. Ann Arbor Staging System

A staging system is a way for members of the cancer care team to summarize the extent of a cancer's spread. The staging system most often used to describe the extent of non-Hodgkin lymphoma in adults is called the Ann Arbor staging system.

The stages are described by Roman numerals I through IV (1-4). Lymphomas that affect an organ outside of the lymph system (an extranodal organ) have E added to their stage (for example, stage IIE), while those affecting the spleen have an S added.

Stage I

Either of the following means the disease is Stage I:

- The lymphoma is in only 1 lymph node area or lymphoid organ such as the thymus (I).
- The cancer is found only in 1 area of a single organ outside of the lymph system (IE).

Stage II

Either of the following means the disease is Stage II:

- The lymphoma is in 2 or more groups of lymph nodes on the same side of (above or below) the diaphragm (the thin band of muscle that separates the chest and abdomen). For example, this might include nodes in the underarm and neck area but not the combination of underarm and groin nodes (II).
- The lymphoma extends from a single group of lymph node(s) into a nearby organ (IIE). It may also affect other groups of lymph nodes on the same side of the diaphragm.

Stage III

Either of the following means the disease is Stage III:

- The lymphoma is found in lymph node areas on both sides of (above and below) the diaphragm.
- The cancer may also have spread into an area or organ next to the lymph nodes (IIIE), into the spleen (IIIS), or both (IIISE).

Stage IV

Either of the following means the disease is Stage IV:

- The lymphoma has spread outside of the lymph system into an organ that is not right next to an involved node.
- The lymphoma has spread to the bone marrow, liver, brain or spinal cord, or the pleura (thin lining of the lungs).

Other modifiers may also be used to describe the lymphoma stage:

Bulky Disease

This term is used to describe tumors in the chest that are at least one-third as wide as the chest, or tumors in other areas that are at least 10 centimeters (about 4 inches) across. It is usually designated by adding the letter X to the stage. Bulky disease may require more intensive treatment.

A vs. B

Each stage may also be assigned an A or B. The letter B is added (stage IIIB, for example) if a person has any of the B symptoms listed below:

- Loss of more than 10% of body weight over the previous 6 months (without dieting)
- Unexplained fever of at least 101.5°F
- Drenching night sweats

These symptoms usually mean the disease is more advanced. If a person has any of these, then more intensive treatment is usually recommended. If no B symptoms are present, the letter A is added to the stage.

Appendix B. Karnofsky Performance Status Scores

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

Appendix C. Cockcroft-Gault Calculation for Creatinine Clearance

$$CrCL = \frac{[(140 \quad \text{Age}) * \text{Body Mass (in kg)}]}{[72 * \text{Serum Creatinine (in mg/dL)}]}$$

If the patient is female, multiply the above by 0.85. If the patient is obese (> 30% over ideal body weight), use ideal body weight in calculation of estimated CrCL.

Appendix D. Amgen ISS FAX Transmittal Form - Carfilzomib

Investigator Sponsored Study

Amgen ISS

FAX Transmittal Form

Carfilzomib

20159860 / IST-CAR-591 / Phase I/Ib Study of Carfilzomib plus Rituximab plus Ifosfamide plus Carboplatin plus Etoposide (C-R-ICE) in Patients with Relapsed/Refractory Diffuse Large B-cell Lymphoma (DLBCL)

To: Amgen Global Safety

Toll-free #:1-888-814-8653

Email *(Only for sponsors with a secure email connection with Amgen):*

svc-ags-in-us@amgen.com

AMGEN ISS PROTOCOL

#: 20159860 / IST-CAR-591

Sponsor: Roswell Park

Sponsor Contact Name: _____

Fax No: _____

Phone No:_____

Date: _____

Use this form as a cover page for an individual report, for batched individual reports, and for line listings.

****NOTE: Please use data reconciliation fax cover sheet to submit data reconciliation line listings.***

Fax transmission contents (Check all that apply):

To be sent immediately after each single case submission to RA, EC, IRB or DMC:

☐ Expeditable Serious Adverse Events/Serious Adverse Drug Reactions

# of Reports Submitted: _____

To be sent in regular intervals per contractual agreement (eg, as batched individual reports or line listings):

☐ Serious Adverse Drug Reactions **#** of Reports Submitted: _____

Period from _____ to _____

DDMMM/YYYY DD/MMM/YYYY

☐ Serious Adverse Events **#** of Reports Submitted: _____

*(does **NOT** apply for marketed Amgen products)*

Period from _____ to _____

DD/MMM/YYYY DD/MMM/YYYY

Other Reports (to be sent as per contractual agreement eg, pregnancy/lactation reports)

☐ Specify type of report: _____ **#** of Reports Submitted: _____

Period from _____ to _____

DD/MMM/YYYY DD/MMM/YYYY

Total # of pages in this transmission, including cover page: _____

Appendix E Instructions for Network Sites

1. CONTACT INFORMATION

All questions related to the protocol or study implementation should be directed to:

Roswell Park Cancer Institute
CRS Network Office
ASB K 104
Buffalo, New York 14263
Telephone:
Monday Friday; 8: 00 AM to 4: 30 PM EST
716-845-3870
After hours, weekends, and holidays request the RPCI Investigator
716-845-2300
Fax: 716-845-8743

2. INFORMED CONSENT

Informed consent must be obtained by the site Investigator/designee from any participants wishing to participate, prior to any study specific procedures.

An informed consent template is provided by RPCI and can be amended to reflect institutional requirements.

All consent changes must be reviewed by RPCI Network Office prior to submission to the site IRB.

The informed consent must be IRB approved.

Always check that the most up to date version of the IRB approved consent is being used.

Within 5 business days, notify the RPCI Network Office of all participant withdrawals or consent to limited study participation and appropriately document the discontinuation and the reason(s) why.

3. PARTICIPANT REGISTRATION

The participant completes the Gender, Race, and Ethnicity Form and this is placed in the study binder.

RPCI does not grant exceptions to eligibility criteria.

Phase 1 Protocol Registration Instructions

Contact the RPCI Network Monitor to verify that a slot is available in the open cohort when a participant has been identified. Do not have the participant sign consent prior to verifying an open slot.

- **After the participant signs consent, the Subject Screening and Enrollment Log must be faxed or emailed to the RPCI Network Monitor within 1 business day. The RPCI Network Monitor will confirm receipt of the Subject Screening and Enrollment Log and email the participant ID number.**

- **When the participant has met eligibility, a signed eligibility checklist and other requested documentation will be faxed or emailed to the RPCI Network Monitor.**

- **Within 1 business day of receipt of the eligibility check list, the RPCI Network Monitor will fax or email the cohort assignment and dose level.**

- **An email must be sent by the site to confirm receipt of the cohort assignment and to provide the planned treatment start date.**

4. STUDY DEVIATIONS

If a deviation has occurred to eliminate hazard, this must be reported to the RPCI Network, site IRB and any other regulatory authority involved in the study.

ALL study deviations will be recorded on the Study Deviation Log.

Participants inadvertently enrolled with significant deviation(s) from the study-specified criteria will be removed from the study, at the discretion of the Principle Investigator.

5. STUDY DOCUMENTATION

Study documents must be filled out completely and correctly. Ditto marks are not allowed.

If an entry has been documented in error put a single line through the entry and initial and date the change. The RPCI Network Monitor must be able to read what has been deleted.

Do NOT use white-out, magic marker, scratch-outs.

Do NOT erase entries.

Use only black ink for documentation on the accountability form and any other study forms.

It is the responsibility of RPCI to inform the Investigator/ institution as to when these documents no longer need to be retained. If, for any reason, the Investigator desires to no longer maintain the study records, they may be transferred to another institution, another investigator, or to RPCI upon written agreement between the Investigator and RPCI.

6. DRUG ACCOUNTABILITY

Drug accountability must be strictly maintained.

Responsibility rests solely with the Investigator but can be delegated as appropriate (e.g., to pharmacy personnel).

A drug accountability record form (DARF) will record quantities of study drug received, dispensed to participants and wasted, lot number, date dispensed, participant ID number and initials, quantity returned, balance remaining, manufacturer, expiration date, and the initials of the person dispensing the medication.

Study drug supply will only be used in accordance with the IRB approved study.

Drug accountability forms are protocol and agent specific, they are study source documents and will be used to verify compliance with the study.

An inventory count must be performed with each transaction. Any discrepancies shall be documented and explained.

Drug accountability forms must be stored with study related documents.

Each medication provided for this study and each dosage form and strength must have its own DARF.

Dispensing the wrong study supply is considered a medication error.

NEVER replace investigational agents with commercial product.

Do NOT "transfer", "borrow" or "replace" supplies between studies.

7. SERIOUS ADVERSE EVENT REPORTING

The site Investigator or designated research personnel will report all SAEs, whether related or unrelated to the investigational agent(s) to the IRB in accordance with their local institutional guidelines. The site will notify the RPCI Network Monitor within 1 business day of being made aware of the SAE. A preliminary written report must follow within 1 business day of the first notification using the following forms:

RPCI SAE Source form

MedWatch 3500A

See section 11 for additional reporting information

A complete follow-up report must be sent to the RPCI Network Monitor when new information becomes available.

8. UNANTICIPATED PROBLEM REPORTING

An unanticipated problem (UP) is any incident, experience, or outcome that meets all of the criteria in Section 11.5.

For all adverse events occurring that are unanticipated and related or possibly related to the research drug, biologic or intervention, the participating physician or delegated research staff from each site will notify their local IRB in accordance with their local institutional guidelines. The site must also notify the RPCI Network Monitor within 1 business day of being made aware of the Unanticipated Problem by completing the RPCI Unanticipated Problem Report Form and faxing or emailing it to the RPCI Network Monitor.

APPENDIX F Roswell Park Cancer Institute Form

Specimen Shipment Form **Study Number: I 240813**			
Patient ID: **2408VU_ _**			**Fax a copy to:** **Cory Mavis** **716-845-7188**
Date Collected:			
Specimen Type:			
Specimen Site:			
Note: Specimen must be shipped between Mondays to Thursdays Please schedule surgical procedures accordingly to adhere to shipment days. Ship via overnight mail (check AM delivery)			
FedEx the Original form with the specimen to: Roswell Park Cancer Institute Department of Medicine Lymphoma/Myeloma Laboratory CCC Bldg. 3rd Floor, Rm 304 Attn: Study Number – I 201611 Elm & Carlton Streets Buffalo, NY 14263 Tel: 716-845--3464 Fax: 716-845-7188 Contact: cory.mavis@RoswellPark.org			
PLEASE INDICATE THE APPROPRIATE SAMPLE BEING SHIPPED			

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