Carfilzomib, Rituximab and Dexamethasone (CaRD) is active and offers a neuropathy-sparing approach for proteasome-inhibitor based therapy in Waldenström’s Macroglobulinemia.

Steven P. Treon¹,², Christina K. Tripsas¹, Kirsten Meid¹, Sandra Kanan¹, Patricia Sheehy¹, Stacey Chuma¹, Lian Xu¹, Yang Cao¹, Guang Yang¹, Xia Liu¹, Christopher J. Patterson¹, Diane Warren¹, Zachary R. Hunter¹, Barry Turnbull³, Irene M. Ghobrial¹,², Jorge J. Castillo¹,².

¹Bing Center for Waldenstrom’s Macroglobulinemia, Dana-Farber Cancer Institute, Boston, MA; ²Harvard Medical School, Boston, MA; BioBridges Inc., Newton MA.

Corresponding author:

Steven P. Treon M.D., M.A., Ph.D.
Bing Center for Waldenström’s Macroglobulinemia
Dana Farber Cancer Institute
Harvard Medical School
M548, 450 Brookline Avenue
Boston MA 02215 USA
Tel: (617) 632-2681
Fax: (617) 632-4862
Email: steven_treon@dfci.harvard.edu

Short Title: Carfilzomib, Rituximab, Dexamethasone in Waldenstrom’s
Keywords: Waldenström’s Macroglobulinemia, Carfilzomib, Rituximab, Dexamethasone, MYD88, CXCR4.
Keypoints

- Carfilzomib, Rituximab and Dexamethasone (CaRD) produces overall and CR/VGPR responses in 87% and 36% in frontline WM patients.
- CaRD activity was not impacted by MYD88 and CXCR4 mutations, and represents a neuropathy-sparing option for treating WM patients.

Abstract

Bortezomib frequently produces severe treatment-related peripheral-neuropathy in Waldenstrom’s Macroglobulinemia (WM). Carfilzomib is a neuropathy-sparing proteasome-inhibitor. We examined carfilzomib, rituximab, and dexamethasone (CaRD) in symptomatic WM patients naïve to bortezomib and rituximab. Protocol therapy consisted of IV carfilzomib 20 mg/m² (cycle 1), 36 mg/m² (cycles 2-6) with IV dexamethasone 20 mg on days 1,2,8,9 and rituximab 375 mg/m² on days 2,9 every 21-days. Maintenance-therapy followed 8-weeks later with IV carfilzomib 36 mg/m² and IV dexamethasone 20 mg on days 1,2 and rituximab 375 mg/m² on day 2 every 8-weeks for 8-cycles. Post-therapy, median serum IgM levels declined from 3,375 to 749 mg/dL (p<0.0001); bone marrow disease declined from 60% to 5% (p<0.0001); hematocrit rose from 32.3% to 41.3% (p<0.0001). Overall response rate was 87.1% (1 CR, 10 VGPR, 10 PR, 6 MR), and was not impacted by MYD88L265P or CXCR4WHIM mutation status. With a median follow-up of 15.4 months, 20 patients remain progression-free, including 8 on maintenance-therapy. Grade >2 toxicities included asymptomatic hyperlipasemia (41.9%), reversible neutropenia (12.9%), and cardiomyopathy in one patient (3.2%) with multiple risk factors. Declines in serum IgA and IgG were common. CaRD offers a neuropathy-sparing approach for proteasome-inhibitor based therapy in WM. Study registered at www.clinicaltrials.gov (NCT01470196).
Introduction

The proteasome inhibitor bortezomib is active in Waldenstrom’s Macroglobulinemia (WM), with single agent responses in 60-70% of untreated or relapsed/refractory WM patients, and response durations of 8-16 months. In combination with rituximab and dexamethasone, an overall response rate (ORR) of 95% was observed for untreated patients in a study by the Waldenstrom’s Macroglobulinemia Clinical Trials Group (WMCTG). Very good partial responses (VGPR) or better occurred in 35% of patients, and the median progression-free survival (PFS) exceeded 4 years. Unfortunately, 70% of patients developed grade ≥2 peripheral neuropathy (PN) with the twice weekly dosing of bortezomib in this study, and resulted in premature bortezomib discontinuation in 60% of patients.

The use of weekly bortezomib represented one of the first attempts to decrease PN risk in WM. Grade 3 PN occurred in 5-20% of patients, and resulted in bortezomib discontinuation in 10-20% of subjects. Lower rates of VGPR or better accompanied weekly bortezomib use in some studies, a finding with possible implications for long-term disease control. In a hybrid study by the European Myeloma Network (EMN), patients received twice weekly bortezomib for the first cycle, then weekly bortezomib for cycles 2-5 with dexamethasone and rituximab. An ORR of 85%, with VGPR or better in 10% of patients was observed. The median PFS was 43 months, and patients with VGPR/CR had longer PFS. Grade ≥2 treatment-related PN occurred in 24% of patients, and led to bortezomib discontinuation in 8% of patients.
Treatment-related PN with bortezomib can persist long after discontinuance of therapy, and often requires the chronic use of gabapentin, pregabalin and/or narcotics. The higher incidence of bortezomib related PN in WM may reflect underlying nerve damage due to paraprotein-mediated demyelination and/or amyloid deposition that commonly occurs in WM.\textsuperscript{11,12} Carfilzomib is a second-generation proteasome-inhibitor with a low neuropathic risk, including patients with baseline PN.\textsuperscript{13} Differences in off-target (non-proteasome effects) between bortezomib and carfilzomib have been proposed to account for the lower neuropathic potential of carfilzomib.\textsuperscript{14} \textit{In vitro}, carfilzomib selectively inhibits the chymotrypsin-like activity of both the constitutive- and immunoproteasome, the later exhibiting higher expression in WM versus normal B-cells, and representing a selective target for WM cell killing.\textsuperscript{15} As such, we evaluated the combination of carfilzomib, rituximab and dexamethasone (CaRD) in patients with symptomatic WM. We also evaluated the influence of MYD88\textsuperscript{L265P} and CXCR4\textsuperscript{WHIM} somatic mutation status on response outcome given their high prevalence in WM patients, association with WM disease related clinical features, and potential impact on treatment response.\textsuperscript{16-18}
Patients and Methods

This was a prospective, open-label, single stage, phase II study which enrolled patients at the Dana Farber Cancer Institute, Boston MA. All patients provided informed written consent and the Institutional Review Board of the Harvard/Dana Farber Cancer Center granted study approval. The study was conducted in accordance with the Declaration of Helsinki. Carfilzomib was supplied by Onyx Pharmaceuticals, Inc. Rituximab and dexamethasone was commercially obtained. The primary objective of the study was the determination of overall response rate (i.e. complete response [CR], very good partial response [VGPR], partial response [PR] and minimal response [MR] rates). Secondary objectives were determination of time to progression (TTP), as well as safety and tolerability including incidence of treatment peripheral neuropathy following CaRD therapy.

Symptomatic WM patients requiring therapy based on consensus recommendations, who were previously untreated with a proteasome-inhibitor or rituximab, and had no more than one prior therapy were eligible. Intended therapy consisted of intravenous (IV) carfilzomib 20 mg/m² infused over 20 minutes (cycle 1 only), then 36 mg/m² infused over 30 minutes (for cycles 2 and beyond) with IV dexamethasone 20 mg given on days 1, 2, 8, 9 and rituximab 375 mg/m² on days 2, 9 every 21 days for 6 cycles of induction. Induction for patients with stable disease or better was followed by maintenance therapy 8 weeks later with IV carfilzomib 36 mg/m² and IV dexamethasone 20 mg given on days 1, 2 along with rituximab 375 mg/m² on day 2 only every 8 weeks for 8 cycles.
Rituximab was administered after carfilzomib and dexamethasone. For the first cycle only, treatment was administered after one liter of saline to prevent azotemia. For grade >3 non-hematological toxicity, carfilzomib was held until toxicity was grade 1 or less, with dose reduction then permitted to 27 mg/m² (level -1) for the first event and 20 mg/m² (level -2) for the second event after cycle 1. For hematological toxicities, carfilzomib was held for an ANC <500/mm³ or platelet count <30,000/mm³. Filgrastim or transfusional support was permitted. Retreatment at the full dose of carfilzomib was permitted after the first occurrence of a drug hold for hematological toxicity, but thereafter reduction to level -1 for the second event, and level -2 for the third event was required. For grade ≥2 treatment related neuropathy with pain, or grade 3 neuropathy without pain, treatment was held until neuropathy resolved to less than grade 2 without pain, followed by decrease in carfilzomib to level -1 for the first event, and level -2 for the second event. For grade 4 neuropathy, carfilzomib discontinuance was required.

For patients experiencing rituximab or dexamethasone intolerance, their discontinuation was permitted. Concomitant medications included acyclovir 400 mg twice a day for the duration of therapy plus 6 months for herpes zoster prophylaxis, and famotidine 20 mg twice a day during weeks of active therapy for prophylaxis against dexamethasone-related gastrointestinal toxicity. Dexamethasone (10 mg) was recommended the night before rituximab administration to attenuate risk of infusion reactions. The use of green tea or extracts was discouraged given potential adverse interactions with proteasome inhibitors.²⁰ As per consensus and NCCN guidelines, prophylactic use of plasmapheresis and hold on rituximab administration for patients demonstrating an IgM
Baseline studies consisted of complete blood counts and differential (CBCD), quantitative serum IgM levels, serum protein electrophoresis (SPEP), a BM biopsy and aspiration, computed tomography (CT) scans of the chest, abdomen and pelvis (CAP), serum electrolytes, liver function tests (LFTs), amylase, lipase, blood urea nitrogen (BUN), creatinine, and serum β₂-microglobulin levels. Patients were assessed for efficacy and toxicity on first day of induction cycles 2-6; at interphase which occurred within 4 ± 2 weeks after end of induction therapy; on first day of maintenance cycles 1-8; at conclusion of maintenance therapy which occurred with 4 ± 2 weeks following last maintenance cycle; and thereafter off therapy every 12 ± 2 weeks. Required laboratories included CBCD, quantitative serum IgM levels, SPEP, LFTs, amylase, lipase, BUN, and creatinine on day 1 of each treatment cycle, interphase, end of maintenance, and during follow-up visits. At interphase and end of maintenance visits, a BM biopsy and aspiration, and CT scans of CAP if extramedullary disease was present at baseline were required.

**MYD88L265P and CXCR4WHIM Mutation Genotyping**

An allele specific polymerase chain reaction (PCR) assay was used for determination of MYD88L265P using DNA isolated from CD19-selected BM or peripheral blood (PB) cells as previously described.23,24 CXCR4WHIM mutation status was determined by Sanger sequencing of CD19-selected BM cells as before.16,17
Statistical analysis

It was hypothesized that the observed overall response rate (ORR) would be approximately 70% based on assumptions derived from previous published experiences with frontline bortezomib combination regimens. A single-sided lower 95% confidence bound with a sample size of 30 and an ORR of 70% (i.e. 21 responses out of 30 patients) would equal 53.5% when the exact binomial method was employed. If the obtained ORR single-sided lower 95% confidence interval (CI) was less than 40% further study of the regimen in this indication would be deemed not warranted. Sixteen or fewer responders out of 30 patients (ORR < 56.7%) would result in a single-sided lower ORR CI of less than 40%.

Response determinations were made using consensus criteria adopted from the Sixth International Workshop on WM. Complete response (CR) was defined as resolution of all symptoms, normalization of serum IgM levels with complete disappearance of IgM paraprotein by immunofixation, absence of BM disease, and resolution of any adenopathy or splenomegaly. Patients with very good partial (VGPR), partial (PR) and minor (MR) responses were defined as a ≥ 90% or normalization of serum IgM, ≥ 50%, and 25-49% reduction in serum IgM levels, respectively. Progressive disease (PD) was defined as greater than 25% increase (with an absolute 500 mg/dL) in serum IgM from nadir with reconfirmation required, in the absence of a suspected rituximab induced flare, or progression of clinically significant disease-related symptoms.
TTP was defined as the time between initiation of therapy and date of progression, death or last follow-up. For time to event analyses with censoring, the Kaplan–Meier method was used to estimate survival curves, which were compared using the log-rank test. The resulting hazard ratio (HR) was presented with 95% confidence interval (CI). A Wilcoxon Rank-Sum test was used for analysis of pre- and post-therapy continuous variables. For categorical variables such as response versus non-response, a two-tailed Fisher’s exact test was used. P-values < 0.05 were considered statistically significant. All graphics and calculations were obtained using STATA 13.1 (StataCorp LP, College Station, TX, USA).
Results

Patients and disease characteristics

Thirty-one patients were enrolled which included one patient above the intended study sample who was consented simultaneously with subject number 30 and allowed to participate following IRB approval. The baseline characteristics for these patients are shown in Table 1. The International Scoring System for WM (ISSWM) score for these patients was low (11; 35.5%); intermediate (15; 48.4%), and high (5; 16.1%).\(^{23}\) MYD88 and CXCR4 somatic mutation status was evaluable in 30 patients. Twenty-nine (96.6%) patients demonstrated the MYD88\(^\text{L265P}\) mutation, and 11 (36.7%) had CXCR4\(^\text{WHIM}\) mutations, including 7 with non-sense C-terminal truncating mutations, and 4 with C-terminal frameshift mutations. Twenty-eight (90.3%) patients were previously untreated, and three patients received everolimus on a clinical protocol. Reasons (multiple causes can apply) for treatment initiation based on WM consensus criteria were symptomatic anemia (n=30), extramedullary disease (n=5), hyperviscosity (n=4), and IgM-related PN (n=3). The median number of treatment cycles administered including induction and maintenance was 12 (range 4-14). Ten patients completed all 14 cycles of planned induction and maintenance therapy, and 8 patients are currently receiving maintenance therapy. Protocol therapy was truncated for cardiomyopathy (n=1), progressive IgA/IgG hypogammaglobulinemia and recurring sinobronchial infections (n=2), and for non-response or progression (n=10).
Response

Median IgM levels for all 31 patients declined from 3,375 (range 345-7,430 mg/dL) at baseline to 749 (range 22-4,540 mg/dL) at best response (p<0.0001). Pre-therapy, 20/31 (64.5%) patients demonstrated a serum IgM level > 3,000 mg/dL; following treatment, 4 of 31 (12.9%) patients had a serum IgM level > 3,000 mg/dL (p<0.0001). Median BM involvement decreased from 60% (range 10-95%) to 5% (range 0-95%) at best response (p<0.0001), while hematocrit rose from a median of 32.3% (range 24.1-38.0%) to 41.3% (range 30.9-47.8%) at best response (p<0.0001). Serum IgM and hematocrit levels during the course of active therapy are shown in Figure 1. Categorical responses were as follows: CR (n=1); VGPR (n=10); PR (n=10); MR (n=6); for an ORR and major response (PR or better) rate of 87.1% (95% CI 75.3-98.9%) and 67.7% (95% CI 51.2-84.2%), respectively. A CR/VGPR was achieved in 36% of patients. Overall (p=0.633), major (p=0.776), and VGPR/CR (p=0.785) attainment was not impacted by ISSWM score. Nearly all (96.6%) genotyped patients expressed the MYD88L265P somatic mutation. The one patient with MYD88 wild-type achieved a VGPR. CXCR4WHIM mutations were present in 11/30 (36.7%) study participants. The ORR for patients with CXCR4 wild-type and WHIM mutations were 85% and 90.9%, respectively (p=1.0). Attainment of PR or better occurred in 65% and 73% of patients with CXCR4 wild-type and WHIM mutations, respectively (p=0.711). No significant differences in ORR or major responses were observed between patients with non-sense or frameshift CXCR4WHIM mutations (data not shown). Among the 3 previously treated patients, each of whom
received everolimus prior to study entry, 1 achieved a VGPR; 1 PR; and one was a non-responder who subsequently responded to ibrutinib.

Among responders, the median time to at least a minor response was 2.1 (range 0.7-14.7) months, while the median time to best response was 12.8+ (range 2.1-25+) months. Among 18 patients with CT-defined adenopathy (>1.5 cm), adenopathy either decreased or resolved (n=18; 55.6%), remained stable (n=5; 27.8%) or increased (n=3; n=16.8%) following therapy. Two patients with CT-defined splenomegaly (>15 cm) showed complete resolution after therapy. Three patients had IgM related PN at baseline; 2 showed no change, and one had progression from grade 1 to grade 2 PN that prompted carfilzomib dose reduction to level -1 and no further progression in PN then occurred.

**Time to progression**

With a median follow-up of 15.4 (range 2.1-25.5) months, all patients are alive and 20 (64.5%) remain free of disease progression. The Kaplan-Meier curve for time to progression for all patients appears in Figure 2. Estimation for median time to progression was not possible due to insufficient progression events within the follow-up period. By univariate analysis, BM disease burden (HR 1.03; 95% CI 1.00-1.06; p=0.034) and adenopathy (HR 8.96; 95% CI 1.14-70.3; p=0.037) were associated with increased risk for progression. Categorical response attainment was also associated with risk of progression As shown in Figure 2, patients achieving an MR or PR exhibited
shorter time to progression versus those with VGPR/CR (p<0.001). There were no deaths during the follow-up period, and all patients are alive at this time.

**Toxicities**

Grade 1-4 toxicities that were at least possibly related to protocol therapy are presented in Table 2. The most common Grade > 2 toxicities included dexamethasone-related hyperglycemia (77.4%), carfilzomib-related hyperlipasemia (41.9%) and rituximab-related infusion reactions (19.4%). Hyperglycemia and hyperlipasemia were asymptomatic and reversible in all patients. Hyperlipasemia was often accompanied by hyperamylasemia, and prompted treatment hold (n=6) and/or dose reduction of carfilzomib (n=5). Grade 2 rituximab reactions occurred in 6 patients, and led to rituximab cessation in 3 patients after 2, 9, and 16 infusions. All 3 of these patients progressed during the follow-up period. Grade > 2 neutropenia occurred in 3 (12.9%) patients, and was reversible in all cases. Two patients (one grade 3; one grade 4) required treatment delay and filgrastim until neutrophil recovery. Both patients continued with protocol therapy after neutrophil recovery without any dose reductions or delays. One patient with grade 3 neutropenia had treatment delayed, and recovery of their neutrophil count without filgrastim. Grade > 3 neutropenia events were possibly related to either carfilzomib or rituximab. Anemia related to protocol therapy was rare. One patient who was a non-responder progressed from baseline grade 2 to grade 3, and two patients progressed from grade 1 to grade 2 anemia (both responders), and subsequently normalized. Azotemia occurred in 2 patients (one in cycle 2; one in cycle
5) and resolved after hydration. Grade 2 increases in bilirubin related to carfilzomib occurred in two patients, and were asymptomatic. In one patient, famotidine may have contributed to hyperbilirubinemia, and was discontinued without recurrence. Treatment-related grade 2 PN occurred in one patient with baseline grade 1 disease-related PN without pain, and prompted reduction in carfilzomib to level -1. This patient’s neuropathy did not change following reduction of carfilzomib to level -1. Chest pain occurred in one patient, and extensive workup suggested a likely musculoskeletal etiology for this event. Cardiomyopathy triggered cessation of protocol therapy at cycle 4 in one patient. No baseline echocardiogram was available for this patient with heavy alcohol consumption and smoking (>60 pack-years) use. At the time of the event, echocardiogram showed 35-40% ejection fraction, and the patient was in atrial fibrillation. Extensive cardiological workup was unrevealing including for cardiac amyloidosis. The patient’s atrial fibrillation resolved after diuresis, and repeat echocardiogram showed stable ejection fraction. The patient subsequently discontinued alcohol and smoking consumption and remained asymptomatic without progression off-protocol therapy. A repeat echocardiogram obtained 6 months later showed a normal ejection fraction.

**Rituximab-induced IgM flare**

Nine patients underwent prophylactic pre-therapy plasmapheresis, which included 4 patients for whom omission of rituximab occurred for at least one cycle. Of the 22 patients who did not require prophylactic plasmapheresis, a flare in serum IgM level (≥25%) associated with rituximab was observed in 5 (22.7%) patients and prompted plasmapheresis in one patient.
IgA and IgG hypogammaglobulinemia

At baseline, median serum IgA and IgG levels were 47 and 715 mg/dL, respectively, with 21 (67.7%) and 15 (48.4%) patients demonstrating IgA and IgG hypogammaglobulinemia. Following therapy, median serum IgA and IgG levels declined to 23 and 238 mg/dL (p=0.002 and p<0.0001, respectively), with 28 (90.3%) and 28 (90.3%) patients demonstrating IgA and IgG hypogammaglobulinemia (p=0.058 and 0.0003, respectively). Serum IgA and IgG levels for all patients during the course of therapy are shown in Figure 3. Protocol therapy was truncated after maintenance cycle 7 in two patients with recurring sinobronchial infections associated with treatment-aggravated IgG hypogammaglobulinemia, and for whom intravenous gammaglobulin (IVIG) therapy was initiated. Two other patients required IVIG for treatment aggravated IgG hypogammaglobulinemia and recurring sinobronchial infections, but continue on protocol therapy.
Discussion

Treatment-related PN related to bortezomib is common, and often can be severe and debilitating in WM patients. Carfilzomib is a neuropathy-sparing second generation proteasome-inhibitor. We therefore examined CaRD as an upfront treatment option for WM patients, and observed an ORR of 87.1%. Importantly, 36% of patients achieved a VGPR/CR, including one patient who attained a molecular CR. At baseline, this patient had strongly detectable MYD88\textsuperscript{L265P} positive BM and PB disease with $\Delta$CTs of 1.8 and 3.8, respectively, using a highly sensitive AS-PCR assay. Following maintenance cycle 3 (to confirm CR status), and maintenance cycle 8 (end of therapy visit), MYD88\textsuperscript{L265P} was undetectable in both BM and PB samples using the same AS-PCR assay, thereby constituting the first observation of molecular CR attainment in WM.

Both MYD88\textsuperscript{L265} and CXCR4\textsuperscript{WHIM} mutation status were also evaluated to clarify impact on response attainment. Nearly all patients (96.6%) who were genotyped expressed MYD88\textsuperscript{L265P} precluding a meaningful analysis, though it is interesting that the single patient who was wild-type MYD88 demonstrated a VGPR. No significant differences in either overall or major responses were observed between patients with CXCR4 wild-type or WHIM mutations who received CaRD. The presence of CXCR\textsuperscript{WHIM} somatic mutations was a key determinant for response in a study of relapsed/refractory patients receiving ibrutinib, wherein lower response rates were observed in CXCR4\textsuperscript{WHIM} expressing patients. These findings, though confined to frontline patients, suggest that...
the activity of CaRD is independent of CXCR4\textsuperscript{WHIM} mutation status, and can be observed even with MYD88 wild-type mutation status.

The clinical responses achieved with CaRD were comparable with those achieved using bortezomib in the BDR regimen wherein ORR of 88-95%, and VGPR of 10-35% were observed in upfront patients.\textsuperscript{5,10} The attainment of VGPR/CR is of particular interest given the association of longer PFS in WM patients receiving a rituximab containing regimen, including proteasome inhibitor therapy.\textsuperscript{5,9,10} Consistent with this finding, patients on this study who attained at least a VGPR during the follow-up period demonstrated longer PFS versus patients who attained a minor or partial response. With a median follow-up of 15.4 months, 64% of patients remained free of disease progression, and a longer follow-up will invariably be required to establish the relative PFS for CaRD versus BDR in which PFS of 42 months to >4 years have been reported.\textsuperscript{5,10}

Another important consideration was the relative rapid response time with CaRD. The median time to at least a 25% reduction in serum IgM with CaRD was 2.1 months, which is similar to that reported with BDR (1.5-3 months), and compares favorably against other commonly employed therapies for WM with response times of >3 to 4 months.\textsuperscript{21,26,27} In WM patients in whom more rapid responses may be required, e.g., patients with symptomatic hyperviscosity, cryoglobulinemia, autoimmune cytopenias, etc., CaRD may represent an attractive treatment option.
Overall, treatment with CaRD was well tolerated. Asymptomatic increases in lipase, amylase and bilirubin were common, occurred in 25-50% of patients, and were associated with carfilzomib and dexamethasone administration. The incidence for these events is much higher than reported with other carfilzomib studies, as well as for WM related studies with bortezomib, dexamethasone and/or rituximab.\textsuperscript{5-8,10,13} These events recurred for many patients after treatment with carfilzomib and dexamethasone, and typically resolved by the subsequent cycle. No baseline clinical or disease features correlated with these anomalies, and in one patient, cessation of famotidine led to resolution of recurring hyperbilirubinemia. Reversible grade 2 azotemia occurred in two patients that resolved after hydration. Overall, hematological toxicities were unimpressive with absent or modest levels of grade 3 or higher anemia (3.2%), neutropenia (6.5%) and thrombocytopenia (0%). By comparison, grade > 3 neutropenia (12-15%) and thrombocytopenia (5-8%) were more common, while levels of > grade 3 anemia were similar (0-8%) in WM patients receiving frontline bortezomib combination therapy.\textsuperscript{5,7,10}

Central to this study’s findings was the paucity of treatment-related PN. Only one patient (3.2%) experienced grade 2 treatment related PN, and no patient had discontinuation of CaRD for PN. By comparison, grade 2 or higher PN occurred in 20-70% of WM patients who received upfront bortezomib based therapy, with discontinuation of bortezomib in 10-60% of cases.\textsuperscript{4,5,7,10} In many of these cases, bortezomib related PN continued long after discontinuation of therapy, and necessitated in many patients chronic gabapentin, pregabalin and/or narcotics use.\textsuperscript{11} The high incidence of treatment related PN also limits
prolonged or maintenance use of bortezomib for many patients, thereby limiting the intended therapeutic effects.

Cardiomyopathy occurred in one patient (3.2%) that resulted in premature cessation of intended therapy at induction cycle 4. Cardiomyopathy has been rarely reported in other studies with carfilzomib with an estimated incidence of 7% in a large meta-analysis of 4 studies in relapsed/refractory myeloma patients. Previous exposure to cardiotoxic chemotherapy, including high-dose chemotherapy, could have contributed to these events, and a class effect for proteasome inhibitors might also be relevant since cardiotoxicity has been reported with bortezomib. Smoking, heavy alcohol use and tachyarrhythmia may have also contributed to the single cardiomyopathy event in this study. This patient fully recovered following discontinuation of protocol therapy along with smoking, alcohol consumption, and resolution of tachyarrhythmia. Taken together, these findings necessitate continued pharmaco-vigilance until the relative risk of cardiomyopathy associated with carfilzomib is better understood. Ongoing studies examining bortezomib versus carfilzomib-based therapy in newly diagnosed myeloma patients will be particularly illuminating, and may provide insights into patients at risk for cardiomyopathy. In the interim, WM patients who have risk factors for cardiomyopathy, the use of carfilzomib can be considered against other options, and baseline as well as periodic follow-up echocardiography considered.

IgA and IgG hypogammaglobulinemia are common in WM patients, and can be aggravated with rituximab containing induction and maintenance therapy.
baseline, most patients in this study exhibited IgA and IgG hypogammaglobulinemia, in line with our previous observations. Following CaRD therapy, IgA and IgG levels declined to 48% and 33% of baseline levels, and resulted in treatment truncation at maintenance cycle 7 for two patients who developed recurring sinobronchial infections requiring antibiotic therapy. Close monitoring of IgA and IgG levels should therefore be undertaken in WM patients undergoing CARD therapy, and ongoing maintenance therapy given in context of changes to IgA and IgG levels, and infection risk. Studies examining alternative (i.e. maintenance every 3 months) or truncated (i.e. 6 versus 8 cycles) maintenance schedules should also be considered in future studies to minimize IgA and IgG depletion with CaRD.

In summary, CaRD produces high rates of response including VGPR or better in symptomatic, frontline patients with WM. Responses are rapid, and treatment is well tolerated, with minimal treatment related neuropathy. Patients at risk for cardiomyopathy should be carefully monitored or alternative treatment options considered. Asymptomatic elevations in lipase, amylase and bilirubin levels are common with CaRD, while IgA and IgG depletion occurs in most patients and can be associated with recurring sinobronchial infections. CaRD offers a neuropathy-sparing approach for proteasome inhibitor-based therapy in WM.
Acknowledgements

Supported by the Peter and Helen Bing Fund for Waldenstrom’s Macroglobulinemia at the Dana Farber Cancer Institute, the Linda and Edward Nelson Fund, and Onyx Pharmaceuticals, Inc. who provided study support and carfilzomib for these studies.

Authorship

SPT designed the study. SPT, PS, SK, IG, SC, and JJC provided treatment and follow-up for patients on the study. CT, DW, KM coordinated the study and provided regulatory oversight. CT, KM, CJP and RJM collected the study data. LX, GY, YC, XL, ZRH performed genotyping for MYD88L265P and CXCR4WHIM mutations. SPT, KM, CT, ZRH, BT and JJC analyzed the study data. SPT wrote the manuscript.

Conflict of Interest

Drs. Treon and Ghobrial have received research funding, speaking honoraria and/or consulting fees from Onyx Pharmaceuticals Inc.
References


<table>
<thead>
<tr>
<th></th>
<th>Median</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Age (years)</strong></td>
<td>61</td>
<td>47-75</td>
</tr>
<tr>
<td><strong>Gender (M/F)</strong></td>
<td>19/12</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Serum IgM (mg/dL)</strong></td>
<td>3375</td>
<td>345-7430</td>
</tr>
<tr>
<td><strong>Serum IgA (mg/dL)</strong></td>
<td>47</td>
<td>18-725</td>
</tr>
<tr>
<td><strong>Serum IgG (mg/dL)</strong></td>
<td>715</td>
<td>222-3890</td>
</tr>
<tr>
<td><strong>Hemoglobin (g/dL)</strong></td>
<td>10.7</td>
<td>8.3-13.3</td>
</tr>
<tr>
<td><strong>Hematocrit (%)</strong></td>
<td>32.3</td>
<td>24.1-38.0</td>
</tr>
<tr>
<td><strong>Platelet (mm3)</strong></td>
<td>255,000</td>
<td>68,000-584,000</td>
</tr>
<tr>
<td><strong>β2-microglobulin (mg/L)</strong></td>
<td>3.6</td>
<td>1.9-7.2</td>
</tr>
<tr>
<td><strong>ISSWM Score</strong></td>
<td>2</td>
<td>0-3</td>
</tr>
<tr>
<td><strong>Adenopathy</strong></td>
<td>18 (58.1%)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Splenomegaly</strong></td>
<td>2 (6.5%)</td>
<td>N/A</td>
</tr>
<tr>
<td><strong>Bone Marrow Involvement (%)</strong></td>
<td>60</td>
<td>10-95</td>
</tr>
</tbody>
</table>

**Table 1.** Baseline characteristics for all patients enrolled on study. ISSWM, International Scoring System for WM.23
<table>
<thead>
<tr>
<th>Toxicity Type</th>
<th>Any Grade</th>
<th>Grade 1</th>
<th>Grade 2</th>
<th>Grade 3</th>
<th>Grade 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Anemia</td>
<td>3 (9.7%)</td>
<td>0 (0%)</td>
<td>2 (6.5%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Arthralgia</td>
<td>3 (9.7%)</td>
<td>3 (9.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Azotemia</td>
<td>3 (9.7%)</td>
<td>1 (3.2%)</td>
<td>2 (6.5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Cardiomyopathy</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Chest pain (non-cardiac)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Dyspepsia</td>
<td>1 (3.2%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Fatigue</td>
<td>2 (6.5%)</td>
<td>1 (3.2%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hyperglycemia</td>
<td>31 (100%)</td>
<td>7 (22.6%)</td>
<td>17 (54.8%)</td>
<td>7 (22.6%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hyperamylasemia</td>
<td>8 (25.8%)</td>
<td>7 (22.6%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hyperbilirubinemia</td>
<td>9 (29.0%)</td>
<td>7 (22.6%)</td>
<td>2 (6.5%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hyperkalemia</td>
<td>1 (3.2%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hyperlipasemia</td>
<td>17 (54.8%)</td>
<td>4 (12.9%)</td>
<td>8 (25.8%)</td>
<td>5 (16.1%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Hypokalemia</td>
<td>1 (3.2%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Insomnia</td>
<td>2 (6.5%)</td>
<td>1 (3.2%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Infusion reaction (rituximab)</td>
<td>7 (22.6%)</td>
<td>1 (3.2%)</td>
<td>6 (19.4%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Mucositis</td>
<td>2 (3.2%)</td>
<td>2 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Neutropenia</td>
<td>11 (34.8%)</td>
<td>7 (22.6%)</td>
<td>1 (3.2%)</td>
<td>2 (6.5%)</td>
<td>1 (3.2%)</td>
</tr>
<tr>
<td>Peripheral neuropathy</td>
<td>6 (19.4%)</td>
<td>5 (16.1%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Rash</td>
<td>9 (29.0%)</td>
<td>6 (19.4%)</td>
<td>3 (9.7%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
<tr>
<td>Thrombocytopenia</td>
<td>1 (3.2%)</td>
<td>1 (3.2%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
<td>0 (0%)</td>
</tr>
</tbody>
</table>

Table 2. Adverse events possibly, probably or definitely associated with protocol therapy; (%) denotes number of events.
Figure Legend

Figure 1. Serum IgM (A) and hematocrit (B) levels during the course of protocol therapy including induction cycles (C1-C6), interphase between induction and maintenance therapy (INT), maintenance cycles (M1-M8), and end of treatment (EOT) for all 31 patients who received CaRD. The black line, darker and lighter shaded areas represent the median, interquartile, and range respectively.

Figure 2. Kaplan Meier curve denoting progression free survival for all 31 patients (A) and based on categorical response (B) following treatment with CaRD. 95% confidence interval (CI) for survivor function is shown. (CR, complete response; VGPR, very good partial response; PR, partial response; MR, minor response; NR, no response).

Figure 3. Serum IgA (A) and IgG (B) levels during the course of protocol therapy including induction cycles (C1-C6), interphase between induction and maintenance therapy (INT), maintenance cycles (M1-M8), and end of treatment (EOT) for all 31 patients who received CaRD. The black line, darker and lighter shaded areas represent the median, interquartile, and range respectively.
Figure 2A
Figure 3A
Carfilzomib, rituximab and dexamethasone (CaRD) is active and offers a neuropathy-sparing approach for proteasome-inhibitor based therapy in Waldenström’s macroglobulinemia

Steven P. Treon, Christina K. Tripsas, Kirsten Meid, Sandra Kanan, Patricia Sheehy, Stacey Chuma, Lian Xu, Yang Cao, Guang Yang, Xia Liu, Christopher J. Patterson, Diane Warren, Zachary R. Hunter, Barry Turnbull, Irene M. Ghobrial and Jorge J. Castillo

Advance online articles have been peer reviewed and accepted for publication but have not yet appeared in the paper journal (edited, typeset versions may be posted when available prior to final publication). Advance online articles are citable and establish publication priority; they are indexed by PubMed from initial publication. Citations to Advance online articles must include digital object identifier (DOIs) and date of initial publication.