HHS Public Access

Author manuscript

Clin Cancer Res. Author manuscript; available in PMC 2020 July 01.

Published in final edited form as:

Clin Cancer Res. 2019 July 01; 25(13): 3776–3783. doi:10.1158/1078-0432.CCR-18-1909.

A Phase I/II Trial of Carfilzomib, Pegylated Liposomal Doxorubicin, and Dexamethasone for the Treatment of Relapsed/Refractory Multiple Myeloma

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Abstract

Purpose: Pegylated liposomal doxorubicin (PLD) combined with bortezomib is an effective salvage regimen for relapsed refractory multiple myeloma (RRMM). Carfilzomib, a second-generation proteasome inhibitor, has clinical efficacy even among bortezomib-refractory patients.

Experimental Design: We performed a phase I/II trial of carfilzomib, PLD, and dexamethasone (KDD) with the primary endpoints being safety and efficacy (NCT01246063).

Twenty-three patients were enrolled in the phase I portion and the maximum tolerated dose (MTD) of carfilzomib was determined to be 56 mg/m^2 (Days 1, 2, 8, 9, 15, and 16) when combined with PLD (30 mg/m^2 on Day 8) and dexamethasone (20 mg on Days 1, 2, 8, 9, 15, and 16). Seventeen additional patients were enrolled in the phase II portion.

Results: KDD was determined to be well tolerated with the only common grade 3/4 non-hematologic adverse events of infection. Grade 3/4 hematologic toxicity included lymphopenia (63%), thrombocytopenia (40%), anemia (40%), and neutropenia (28%). In the cohort of patients treated at the MTD, where median prior therapies were 2 and 42% were refractory to bortezomib, the overall response rate was 83% (20/24) with 54% (13/24) having a very good partial response or better. The median progression-free survival was 13.7 months (95% CI 5.0–21.7).

Conclusions: This trial is the first to report outcomes using a triplet regimen of high-dose carfilzomib. KDD was well tolerated and appears efficacious in RRMM. Additional study is

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Conflicts of Interest:

Schroeder: Consultancy/Honorarium (Incyte, Amgen, Sanofi); Fiala: None; Huselton: None; Cardone: Ownership (Eutropics); Jaeger: None; Jean: Ownership (Eutropics); Shea: Ownership (Eutropics); Ghobadi: None; Wildes: Research Founding (Janssen) Honorarium (Carevive); Stockerl-Goldstein: None; Vij: Research Funding (Amgen, Takeda, BMS, Celgene) Ad Boards (Amgen, Takeda, BMS, Celgene, Janssen, Jazz).

Trial Registration: ClinicalTrials.gov # NCT01246063

needed to more precisely determine patient outcomes with this regimen and its utility compared to other carfilzomib containing salvage regimens.

Keywords

carfilzomib; pegylated liposomal doxorubicin; multiple myeloma; mitochondrial profiling; BH3 profiling

INTRODUCTION

The past decade has seen a tremendous growth of available treatment options for relapsed refractory multiple myeloma (RRMM). Carfilzomib was approved by the FDA in 2012 for patients who had received at least two prior therapies including bortezomib and an immunomodulatory agent, based on results of a single-arm multicenter trial of 266 patients.(1) Since that time, numerous clinical trials of novel combination regimens including carfilzomib have been conducted. One notable combination that the literature is currently lacking data on is carfilzomib and pegylated liposomal doxorubicin (PLD).

PLD is FDA approved in combination with bortezomib for RRMM. In the phase III randomized trial that was used to support its indication, PLD was shown to increase progression-free survival (PFS) by approximately 50% over bortezomib alone, but did not show an overall survival advantage in long-term follow up.(2, 3) Carfilzomib is a second-generation proteasome inhibitor (PI) that is an irreversible inhibitor of the 20S proteasome, which is structurally and mechanistically different from bortezomib. It is more selective for the chymotrypsin-like protease, causing less inhibitory activity against other active subunits. The ENDEAVOR trial randomized patients with RRMM to bortezomib and dexamethasone, or carfilzomib and dexamethasone, and demonstrated the superiority of carfilzomib over bortezomib with a median PFS with the carfilzomib regimen at 18.7 versus 9.4 months.(4)

Given the additive or synergistic effect observed with PLD and bortezomib, we hypothesized that the combination of PLD and carfilzomib may be efficacious. To test this, we performed a prospective clinical trial using carfilzomib, PLD, and dexamethasone for RRMM. The study included both a dose escalation phase to determine the maximum tolerated dose (MTD) of the regimen, and an expansion phase powered to determine efficacy. In addition, we tested patient samples collected prior to treatment using a method called BH3 (Bcl-2 homology domain-3) profiling to identify predictive biomarkers for response.

MATERIALS AND METHODS

A single-institution, open-label phase I/II clinical trial of carfilzomib, PLD, and dexamethasone was performed. Carfilzomib and dexamethasone were administered on Days 1, 2, 8, 9, 15, and 16 and PLD was administered on Day 8 of 28 day cycles. Up to 6 cycles of KDD induction treatment were administered. Subsequently, patients continued weekly carfilzomib and dexamethasone maintenance until disease progression or unacceptable toxicity.

The study was performed according to a protocol approved by the Washington University School of Medicine Human Subjects institutional review board (IRB). All subjects provided voluntarily written informed consent for the trial. The study was conducted in accordance with U.S. Common Rule. The study was registered at clinicaltrials.gov as NCT01246063.

Eligible patients were 18 years of age or older with a diagnosis of RRMM post one or more lines of prior treatment and measurable disease in the blood (≥0.5 g/dL by electrophoresis or ≥10 mg/dL difference in involved and uninvolved light-chains), urine (≥200 mg/dL/24 hours), bone marrow (≥30% plasma cells), or extramedullary plasmacytoma. Patients with poor hematologic reserve, liver function, or performance status, and those requiring hemodialysis were excluded, as were patients with a history of plasma cell leukemia, HIV, or active hepatitis. Patients with a left ventricular ejection fraction below 55%, electrocardiogram evidence of acute ischemia or significant conduction system abnormalities, or recent history of acute myocardial infarction, unstable angina, or arrhythmia were excluded due to the known cardiac toxicities of carfilzomib and PLD. Patients who had received prior carfilzomib, PLD or doxorubicin, and those refractory to bortezomib were not excluded.

During the phase I portion of the trial, patients were enrolled in dose escalating cohorts using a standard 3+3 design. Five dose levels were tested with carfilzomib doses ranging from 27 mg/m^2 to 56 mg/m^2. In all cohorts, the Cycle 1 Day 1 and 2 doses of carfilzomib were administered at a lead-in dose of 20 mg/m^2 and then escalated to the cohort specified level for all subsequent doses. All patients received 30 mg/m^2 PLD. The four initial cohorts did not receive dexamethasone to determine the MTD of carfilzomib-PLD doublet-therapy. The fifth cohort received 20 mg of dexamethasone in addition to carfilzomib and PLD at the previously determined MTD. The doses and schedule utilized are detailed in Figure 1.

For MTD determination, dose-limiting toxicity (DLT) was defined as any of the following treatment emergent events related to study treatment during Cycle 1 of induction treatment: grade 4 neutropenia or thrombocytopenia, febrile neutropenia, grade 2 neuropathy with associated pain; grade 3 or higher non-hematologic toxicity; or any other toxicity requiring dose reduction during Cycle 1 or precluding Cycle 2 of treatment on schedule.

Based on *a priori* sample size calculations, we determined that 24 patients treated at the MTD would allow us to determine if the overall response rate (ORR, defined as partial response or better by standard International Myeloma Working Group Criteria) for patients treated with KDD was ≥40%, compared to the null hypothesis that ORR was ≤20%, at 0.1 alpha and 80% power. Therefore, additional patients were enrolled in the phase II expansion cohort until 24 patients total were treated at the MTD.

Correlative Studies

Bone marrow aspirate samples were collected prior to treatment and following discontinuation when possible. We assessed the apoptotic potential of the CD138+ plasma cells using mitochondrial profiling, or BH3 profiling, as a biomarker for identifying patients most likely to respond to study treatment.

Bone marrow samples were viably frozen at the time of collection. During the analytical profiling, the samples were thawed, Ficoll purified, and plasma cells were isolated using anti-CD138 coupled magnetic beads (Miltenyi). BH3 profiling was carried out in an *ex vivo* laboratory developed test by exposing derived plasma samples to peptides comprising the BH3 binding domains of Bcl-2 family proteins and BH3 mimetic compounds. The plasma cells were plated (~10,000 cells per plate) and exposed to varying peptide derivatives from the BH3 family (BIM 100 μM, BIM 0.1 μM, PUMA 10 μM, NOXA 100 μM, BAD 100 μM, HRK 100 μM, BID 0.1 μM or MS-1 50 μM) and with controls dimethyl sulfoxide (1%) or carbonyl cyanide m-chlorophenyl hydrazone (10 μM) upon permeabilization with digitonin and oligomycin. The mitochondria of treated cells were then stained with a fluorescent potentiometric mitochondrial membrane dye, JC-1 (Enzo Life Sciences, Farmingdale, NY, USA), to measure the permeabilization of the mitochondrial outer membrane, which is the key signaling event in the apoptosis cascade. The change in relative fluorescence units (RFU) of the membrane dye compared to the negative and positive controls was continuously recorded using a Tecan Infinite plate reader over a time course. The area under the curve (AUC) of these readouts were determined. The results of each peptide were calculated as percent priming for death, indicating the dependence in a specific signaling pathway. Pearson correlations were used to compare priming of the Bcl-2 family proteins and BH3 mimetic compounds and student's t-test was used to compare KDD responders and non-responders.

RESULTS

Forty patients were enrolled, 23 in phase I and 17 in phase II, from 2012 through 2016. The study was considered complete in November 2017 and the remaining patients on trial at that time were transitioned to additional anti-MM therapy at the discretion of their treating physician.

The baseline characteristics are summarized in Table 1. The median age of the study population was 65 years (range 27–79) and 58% were female. The median number of lines of prior therapy was 2.5 (range 1–13), with all patients receiving prior lenalidomide, 90% bortezomib, 10% carfilzomib, 8% PLD or doxorubicin, and 88% underwent a prior autologous stem cell transplant. Forty-five percent were refractory to bortezomib, 65% to lenalidomide, and 33% were refractory to both.

MTD Determination

No DLTs were observed in the first 4 cohorts tested: [1] 27 mg/m^2 carfilzomib and 30 mg/m^2 PLD, [2]: 36 mg/m^2 carfilzomib and 30 mg/m^2 PLD, [3]: 45mg/m^2 carfilzomib and 30 mg/m^2 PLD, [4]: 56 mg/m^2 carfilzomib and 30 mg/m^2 PLD. Thus, a MTD of doublet-therapy was not reached. Four patients were enrolled into cohorts 2 and 3 as one patient from each was removed from study for disease progression prior to completing the DLT evaluation period. Five patients were enrolled into cohort 4, and as there were no DLTs, it was determined that enrollment would continue with the fifth cohort.

The fifth cohort of patients received KDD triplet-therapy consisting of 56 mg/m^2 carfilzomib, 30 mg/m^2 PLD, and 20 mg of dexamethasone. Seven patients were enrolled,

including one who was removed from the study based on disease progression prior to completing the DLT evaluation period. One of the six evaluable patients experienced grade 4 thrombocytopenia and was considered a DLT. Based on this, the MTD of KDD triplet-therapy was determined to be 56mg/m^2 carfilzomib, 30mg/m^2 PLD, and 20mg of dexamethasone.

Toxicity

Mild to moderate hematologic toxicity was common (Figure 2A); however, severe occurrences were infrequent and were generally treated adequately with growth factor support or transfusion and only resulted in dose reductions in one patient (3%). Grade 3/4 hematologic toxicity included: lymphopenia (63%), thrombocytopenia (40%), anemia (40%), neutropenia (28%), hemolysis (10%), and thrombotic thrombocytopenic purpura (TTP) (3%).

Gastrointestinal upset and constitutional symptoms were common but largely mild (Figure 2B). The rate of palmar-plantar erythrodysesthesia syndrome was 15%. This led to PLD treatment discontinuation in 5%, and PLD dose reductions in another 5%. The incidence of thromboembolic events was 18% and there was one case of reversible posterior encephalopathy syndrome (RPLE). The only common grade 3/4 non-hematologic toxicity was infection (45%) (Figure 2C). Infectious events contributed to the two deaths on study. One patient died of sepsis and one died of acute respiratory failure secondary to H1N1 pneumonia. Both events occurred during cycle 3 of therapy. These events were not considered directly related to study treatment; however, the treatment may have contributed to their immunocompromised state.

As expected with carfilzomib and PLD administration, there were cardiopulmonary toxicities which included: dyspnea (55%), hypertension (33%), hypotension (18%), new or worsened congestive heart failure (5%), and myocardial infarction (3%) (Figure 2D). These events lead to the discontinuation of study treatment in 8% of patients. These events occurred following the DLT observation period in later cycles of treatment.

Efficacy Assessments

Overall, 73% (29/40) of patients treated on the study had a confirmed response, including 40% with a VGPR or better. For determining efficacy, the analysis was limited to patients treated at the MTD. At the MTD, the ORR was 83% (20/24), including 54% (13/24) with a very good partial response (VGPR) or better, and 25% (6/24) obtaining a complete response (CR/stringent CR). The median number of cycles administered was 9.5 (range 1–34). Eleven patients were removed from study due to disease progression. The estimated median PFS, defined as time to progression, was 13.4 months (95% CI 5.0–21.7). Patients who began alternative anti-MM treatment prior to progression were censored. The estimated median overall survival was not reached after a median follow-up of 23.3 months. Efficacy data are summarized in Table 2 and the survival curves are depicted in Figure 3.

Twenty-two patients had received prior bortezomib, 10 were refractory and 12 were sensitive. KDD was effective in patients with bortezomib-refractory disease with 60% (6/10) having an objective response. All bortezomib-responsive/naïve patients responded (4 CR/

sCR, 6 VGPR, and 4 PR). In bortezomib and lenalidomide (double)-refractory patients, the ORR was 50% (3/6). KDD was also effective among patients with high-risk features. The ORR for patients with high-risk disease by ISS (Stage 3) or mSMART criteria was 77% (10/13).

Correlative Studies

Thirty-two patient samples, 28 pre-treatment and 4 post-treatment, met the QC requirements and underwent BH3 profiling. Unless otherwise specified, all data and analyses were limited to the pre-treatment samples. There was a varying degree of correlation between the readout of the assay, “priming” of the various Bcl-2 family proteins determined by exposure to various BH3 mimetic compounds (r^2 = 0.183 – 0.986). Clinical response (partial response or better) was associated with lower priming for three of the analytes. Median NOXA priming was 15.4% among responders compared to 26.2% for non-responders (p=0.0383), PUMA priming 30.5% compared to 43.3% (p=0.013), and HRK priming 20.8% compared to 59.6% (p=0.001) (Figure 4A). Moreover, higher HRK priming was associated with inferior PFS; for each 10 point increase the risk of progression increased by 37% (HR 1.39; 95% CI 1.09–1.72; p = 0.007). Those with HRK priming in the highest quintile (> 40.0%) had a median estimated PFS of 1.8 months compared to 12.4 months for all other patients (p <0.001) (Figure 4B). Three patients had paired pre- and post-treatment samples; all three initially responded to treatment, but later discontinued due to disease progression. There was a trend for increased HRK priming following discontinuation as compared to pre-treatment with a median of 30.5% pre-treatment and 41.8% post-treatment (p = 0.0374) (Figure 4C). HRK priming and all other Bcl-2 family proteins and BH3 mimetic compounds tested were similar between bortezomib-refractory and bortezomib-sensitive/naïve patients (Figure 4D).

DISCUSSION

This phase I/II trial of KDD is the first report of a triplet regimen with high-dose carfilzomib (56 mg/m^2) in RRMM patients. KDD appeared well tolerated and efficacious in RRMM. The estimated ORR of the regimen is 83% (95% CI 68%-98%) with a median PFS of 13.4 months (95% CI 5.0–21.7). The treatment appeared active across high-risk subgroups of patients such as those refractory of bortezomib or with high risk features.

While it is difficult to compare between trials, it is important to interpret results in the context of other carfilzomib containing salvage regimens. The ORR of 83% seen with KDD is comparable to other studies of high-dose carfilzomib including an ORR of 77% with Kd in the ENDEAVOR trial.(4, 5) However, the ORR in subjects that were not refractory to bortezomib was 100% in the current study suggesting the addition of PLD improves response compared to Kd alone. KDD also appeared to elicit deeper responses with a CR/sCR rate of 25% compared to 13% in the ENDEAVOR trial. The ENDEAVOR trial showed a median PFS of 18.7 months compared to 13.4 months with KDD. While the ENDEAVOR trial had a median of 1 prior therapies and only 3% were bortezomib refractory, on the current trial of KDD the median prior therapies was 2 and 42% were refractory to bortezomib. This may account for part of the differences in PFS. In addition, the ENDEAVOR trial continued carfilzomib on the traditional Day 1, 2, 8, 9, 15, and 16

schedule until disease progression. In the current study of KDD, the carfilzomib schedule was reduced to once weekly after cycle 6 for convenience. PLD was also limited to just six cycles based on the potential for cumulative cardiotoxicity from PLD in combination with carfilzomib. The durability of responses in the current study may have been improved had carfilzomib/PLD been continued on the induction schedule.

Another popular carfilzomib triple regimen, carfilzomib (27 mg/m^2), lenalidomide, and dexamethasone (KRd), showed an ORR of 87% with a PFS of 26.3 months in a population with a median of 2 prior therapies and where 15% of patients were refractory to bortezomib and 6% were double-refractory to bortezomib and lenalidomide.(6) In comparison, the bortezomib and double-refractory rates were 42% and 25%, respectively, in the KDD trial. In the KRd trial, carfilzomib was continued on the traditional Day 1, 2, 8, 9, 15, and 16 schedule for 12 cycles. The differences in prior treatments, the additional doses of carfilzomib, and the prolonged exposure to lenalidomide, which was continued until progression, make comparisons difficult.

In addition, a comparison of this regimen to prior reports of PLD and bortezomib combination is relevant.(2) In this prior study, two-thirds of subjects had been treated with ≥2 prior lines of therapy and all subjects were bortezomib naïve. The ORR was 44% (4% CR, and 40% PR) and median PFS was 9 months. The side effect profile of PLD and bortezomib appears similar to KDD but response rates are higher with KDD.

The combination of KDD appeared well tolerated. Grade 3/4 hematologic toxicity included neutropenia (28%), thrombocytopenia (40%), and anemia (40%). This compares to the combination of PLD and bortezomib which reported similar rates of neutropenia (29%), thrombocytopenia (23%), and anemia (9%).(2) The combination of high-dose carfilzomib and dexamethasone alone reported grade 3/4 thrombocytopenia (8%) and anemia (14%).(4) There were, however, cases of hemolysis, TTP, and RPLE suggesting potential for endothelial injury with KDD.

The exact mechanism for endothelial injury is unknown, but others have speculated it could be related to direct effects of proteasome inhibition on NF-kB; or, by causing impairment of vasodilation as well as oxidative and inflammatory stress; or, through an immune mediated mechanism.(7, 8) We believe this effect is independent of PLD and not potentiated by the combination. When compared to safety data from the ENDEAVOR study, the addition of PLD to carfilzomib does increase rates of hematologic toxicity, but there similar rates of non-hematologic toxicity when compared to Kd.(9, 10) This toxicity profile suggests this regimen should be reserved for patients with adequate bone marrow reserve.

Cardiac adverse events are a concern particular to carfilzomib relative to bortezomib with a review of single agent carfilzomib studies showing a 7.2% incidence of cardiac failure.(11) Trials with high-dose carfilzomib reported similar rates of cardiac failure.(4, 12) Combining carfilzomib with an anthracycline may cause concern for an increase in cardiac toxicity, but this was not observed with KDD with only 5% (2/40) of patients having any grade of new or worsening cardiac failure.

A potential next step in the evolution of MM treatment is better selection of the available therapies based on biomarkers predictive of response to treatment. In this study, we analyzed the use of mitochondrial profiling as a prognostic biomarker for response of KDD. The underlying principle of the assay is that aberrant phenotypes in cancer cells lead to dependence on certain Bcl-2 proteins for survival. The assay identifies which protein is involved in cell survival by measuring the ability of various BH3 mimetic proteins to induce apoptosis. These interactions occur primarily through BH3- mediated binding. This indirectly determines the predisposition of that cell to respond to drug induced apoptosis signals and is called “mitochondrial priming.”(13)

Measurements of the mitochondrial priming state have been found to associate with patient response to treatments in MM, AML, DLBCL and CLL.(14–20) These associations have been seen for a range of chemotherapies and targeted drugs including regimens that target anti-apoptotic proteins. The efficacy of the Bcl-2 selective BH3 mimetic compound venetoclax, for instance, is linked to the extent of Bcl-2 dependence.(19)The efficacy of the Mcl-1 targeted BH3 mimetics on the other hand are linked to Mcl-1 dependence.(20) These dependencies can impact any treatment that ultimately relies on mitochondrial apoptosis for efficacy.

Here we have seen that Bcl-xL dependence (high HRK priming), identified in the BH3 profiling assay, was associated with poorer response and inferior outcomes following KDD treatment. It is currently unclear if this is a possible mechanism for resistance to KDD, as detailed in Figure 5, or a nonspecific indicator of more aggressive disease biology. There was a trend of increased priming following disease progression on KDD in the limited number of patients where paired samples were available. However, BH3 profiling was similar in bortezomib-refractory and sensitive/naïve patients prior to KDD. This finding has been previously reported in bortezomib-refractory/naive U266 cell lines.(21)This may suggest that the Bcl-xL dependence is specific to KDD and not a class effect of PIs. Moreover, knowing the HRK score may help screen patients for alternative treatment with Bcl-xL targeted therapies in future studies. The use of BH3 profiling after treatment with such regimens as KDD might also be used to identify survival dependencies and guide the use of Mcl-1 targeted therapies or Bcl-2 targeted therapy like venetoclax.(22, 23)

The strength of this study is its prospective design. The limitations of this study include its non-randomized design and small sample size which make additional analysis such as multivariate infeasible. The original sample size calculation for this study was based on an null hypothesis of <20% ORR, which we concede may be an underestimate given recent studies of monoclonal antibody combinations and small molecule inhibitors showing evidence of higher activity in dual bortezomib and lenalidomide-refractory myeloma patients, but was based on contemporary data at time of protocol design. Despite the limitations, the data suggest that KDD is a viable salvage option beyond second line for patients with MM who are PI exposed or refractory with adequate bone marrow reserve. As monoclonal antibody regimens such as daratumumab + pomalidomide and dexamethasone, are increasingly used in 2nd/3rd line before carfilzomib based regimens, the KDD regimen could provide an alternative to continuing pomalidomide in a successive line of therapy.

In conclusion, in this trial a three-drug regimen of KDD with carfilzomib administered at 56 mg/m^2 was efficacious and well tolerated in patients with RRMM. Biomarkers such as BH3 profiling may help determine which patients are most likely to benefit from the regimen; however, additional study is needed to validate this assay.

ACKNOWLEDGEMENTS

We would like to thank the Alvin J. Siteman Cancer Center at Washington University School of Medicine and Barnes-Jewish Hospital in St. Louis, Missouri, for the use of the Clinical Trials Core which provided protocol development services. We would also like to thank the members of Washington University School of Medicine Division of Oncology Clinical Trials office who provided study coordination and data management.

The Siteman Cancer Center is supported in part by an NCI Cancer Center Support Grant #P30 CA91842. BH3 profiling was supported by NCI-SBIR R44CA203610 awarded to Eutropics.

Funding: Amgen Inc. provided funding and materials for this study

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STATEMENT OF SIGNIFICANCE

A regimen of carfilzomib, pegylated liposomal doxorubicin, and dexamethasone (KDD) is safe, well tolerated, and efficacious in relapsed refractory multiple myeloma.

TRANSLATIONAL RELEVANCE

This trial of carfilzomib 56 mg/m^2, pegylated liposomal doxorubicin (PLD), and dexamethasone (KDD) is the first to report outcomes of a triplet regimen with high-dose carfilzomib for relapsed refractory multiple myeloma. There were no safety concerns and KDD demonstrated an ORR of 83% in heavily pretreated patients including a 60% ORR in bortezomib-refractory patients. KDD compares favorably to other carfilzomib regimens. Furthermore, this paper describes the results of mitochondrial profiling to determine the degree to which each patient's myeloma cells relied on BCL-2 proteins for survival and are "primed" for apoptosis by different BH3 mimetic proteins. This testing suggested BCL-xL dependence was associated with inferior outcomes with KDD. This study suggests mitochondrial profiling may be helpful to guide therapeutic decision making in myeloma.

A

	Carfilzomib	PLD	Dexamethasone
Dose Level 1	27mg/m2	30mg/m2	0mg
Dose Level 2	36mg/m2	30mg/m2	0mg
Dose Level 3	45mg/m2	30mg/m2	0mg
Dose Level 4	56mg/m2	30mg/m2	0mg
Dose Level 5	56mg/m2	30mg/m2	20mg

B

		Day 1	Day 2	Day 8	Day 9	Day 15	Day 16	Day 22
Cycle 1	Carfilzomib	20mg/m^2	20mg/m^2	56mg/m^2	56mg/m^2	56mg/m^2	56mg/m^2	
	PLD			30mg/m^2				
	Dexamethasone	20mg	20mg	20mg	20mg	20mg	20mg	
Cycles 2-6	Carfilzomib	56mg/m^2	56mg/m^2	56mg/m^2	56mg/m^2	56mg/m^2	56mg/m^2	
	PLD			30mg/m^2				
	Dexamethasone	20mg	20mg	20mg	20mg	20mg	20mg	
Cycles 7+	Carfilzomib	56mg/m^2		56mg/m^2		56mg/m^2		56mg/m^2
	PLD							
	Dexamethasone	20mg		20mg		20mg		20mg

Figure 1: Dose Escalation Table and Dosing Schema

[A] Dose escalation [B] Dosing schedule as administered in Phase II

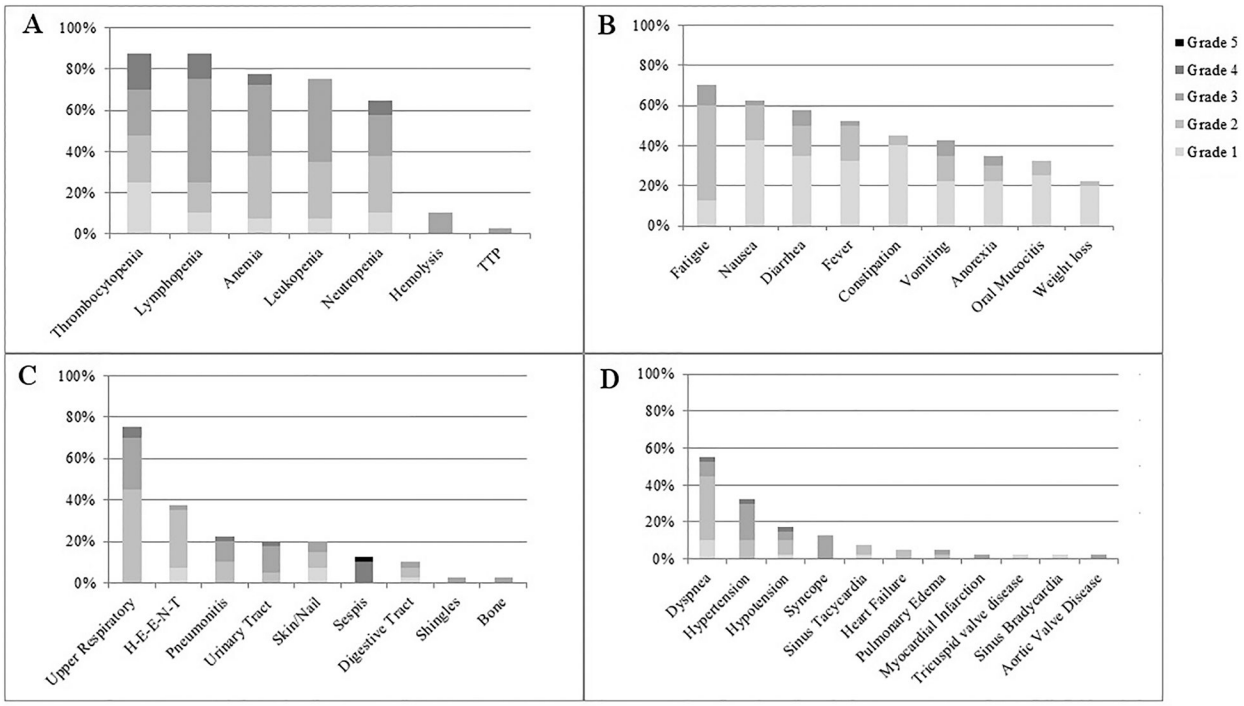

Figure 2: Common Toxicities

The percentage of patients reporting [A] hematologic toxicity, [B] GI upset and constitutional symptoms, [C] infectious complications, and [D] cardiopulmonary toxicities.

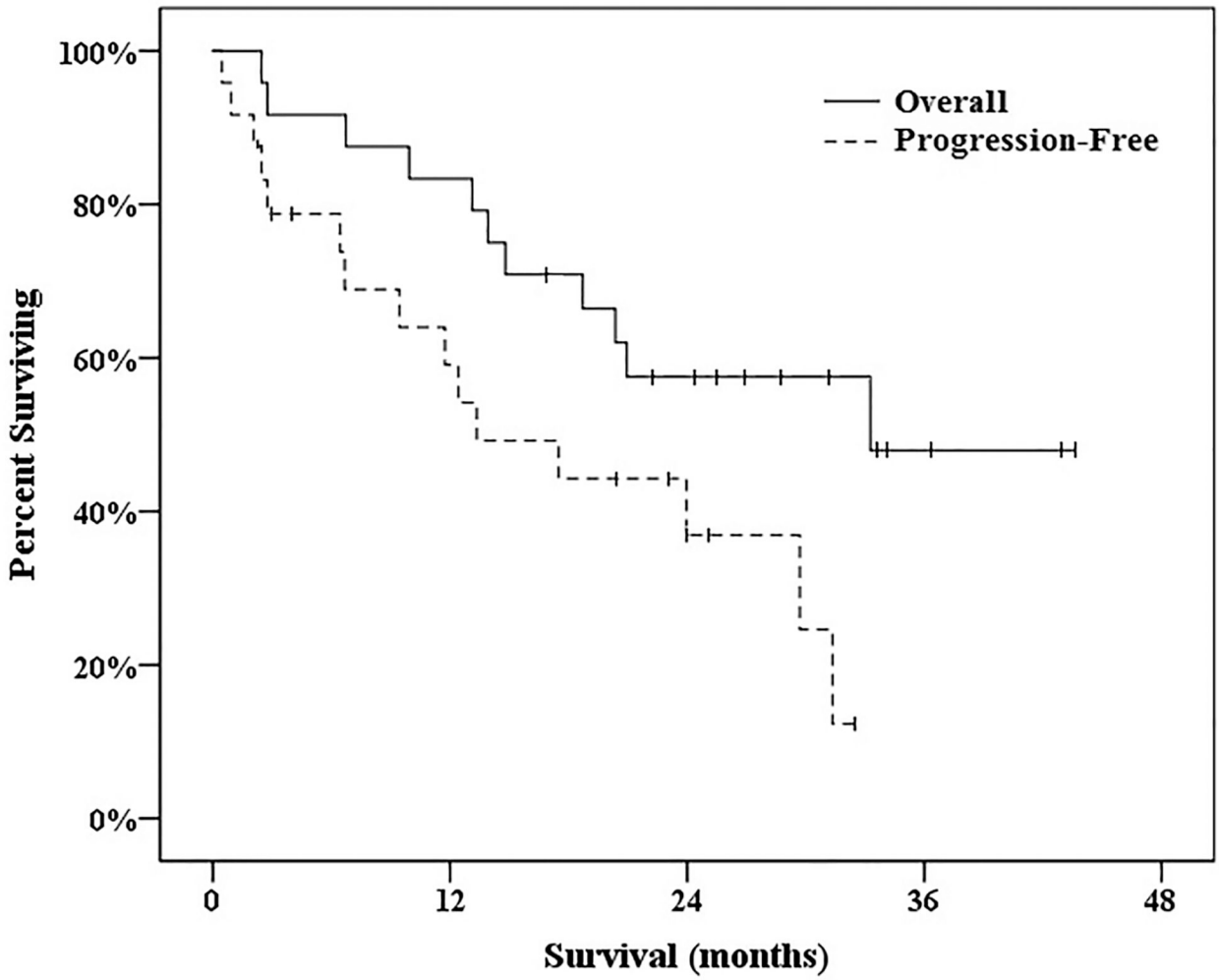

Figure 3: Progression-Free and Overall Survival Curves

The estimated median progression-free survival of patients treated at the MTD was 13.4 months (95% CI 5.0–21.7). Median estimated overall survival has not been reached after a median follow-up of 23.3 months.

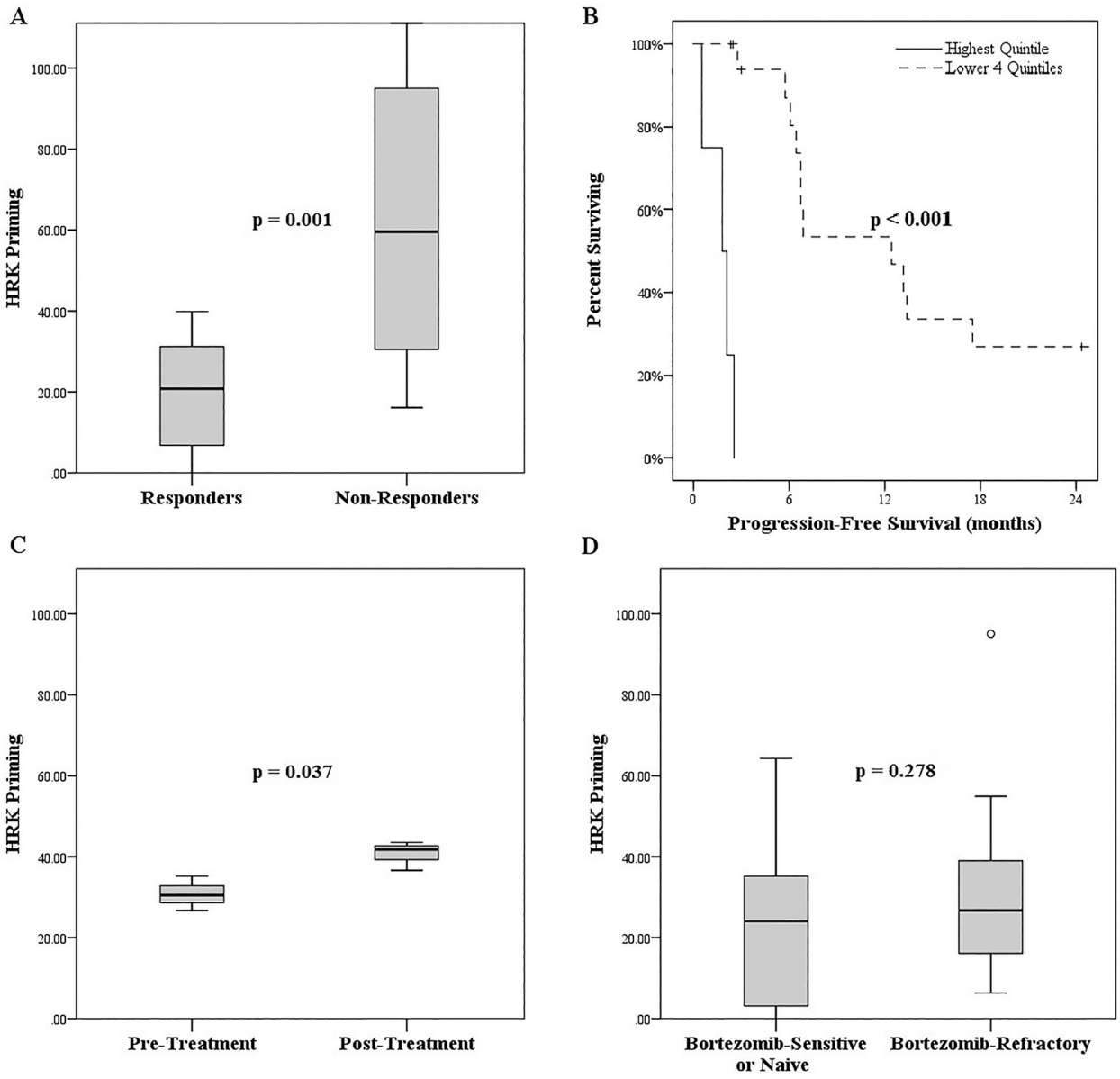

Figure 4: Correlative Studies

[A] HRK priming of responders versus non-responders. [B] Progression-free survival of the highest quintile of HRK expression versus the rest of the cohort. [C] HRK priming pre and post-KDD treatment. [D] HRK Priming in bortezomib-refractory versus bortezomib-sensitive/naïve patients.

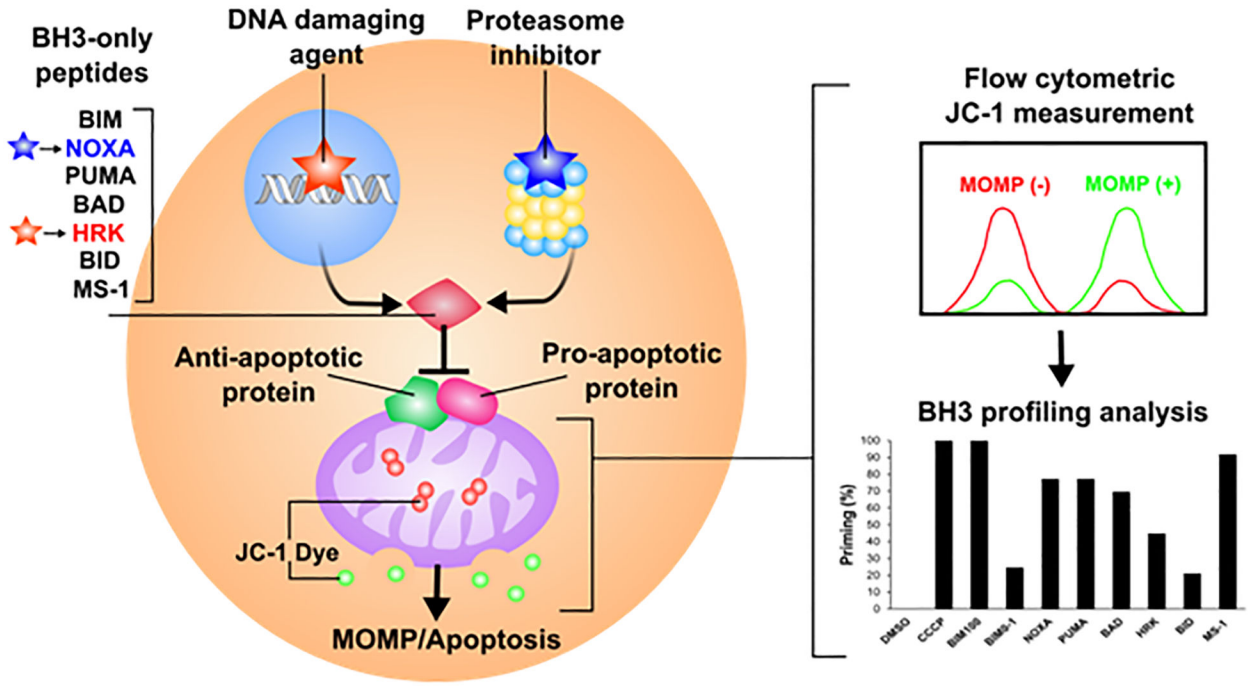

Figure 5: Mitochondrial Priming

DNA damaging (orange) or proteasome inhibiting (blue) drugs illicit apoptosis signaling through select member(s) of BH3-only pro-apoptotic protein family (BIM, NOXA (blue), PUMA, BAD, HRK (orange), BID, and MS-1). These proteins then carry the apoptosis signal to mitochondria, where they impact the free pool of effector pro-apoptotic proteins. When dissociated from anti-apoptotic proteins, the effector proteins cause mitochondrial outer membrane permeabilization (MOMP) triggering the mitochondrial apoptosis signal. In this assay, BH3-only mimetic peptides artificially reproduce this signaling pathway and the MOMP signal from the drug associated BH3 only protein is subsequently analyzed. The extent of MOMP is measured by flow cytometry using a mitochondrial potentiometric dye, JC-1. Thus, BH3 profiling surveys how likely the drugs are to complete the apoptotic signal. This was performed in *ex vivo* experiments at pretreatment as described previously.

Table 1:

Patient Characteristics

	Non-MTD (n=16)	MTD (n=24)	Overall (n=40)
Median Age in Years (range)	66 (53–79)	64 (27–70)	65 (27–79)
Gender			
Male	31%	50%	43%
Female	69%	50%	58%
Race/Ethnicity			
Caucasian	75%	75%	75%
African-American	25%	25%	25%
ISS Stage			
Stage I	31%	46%	40%
Stage II	25%	25%	25%
Stage III	44%	29%	35%
R-ISS Stage			
Stage I	19%	29%	25%
Stage II	63%	63%	63%
Stage III	19%	8%	13%
mSMART Risk *			
Standard Risk	38%	17%	25%
Intermediate Risk	19%	50%	38%
High Risk	44%	33%	38%
Cytogenetic Risk by mSMART criteria			
Standard Risk	56%	33%	43%
Intermediate Risk	19%	58%	43%
High Risk	25%	8%	15%
Treatment History			
Median Prior Therapies (range)	3 (1–12)	2 (1–13)	2.5 (1–13)
Prior ASCT	75%	96%	88%
Bortezomib Exposure/Refractory	88%/50%	92%/42%	90%/45%
Lenalidomide Exposure/Refractory	100%/62%	100%/67%	100%/65%
Double-Refractory	44%	25%	33%
Carfilzomib Exposure/Refractory	6%/0%	13%/0%	10%/0%
PLD or Doxorubicin Exposure/Refractory	6%/0%	8%/0%	8%/0%

Abbreviations: ASCT- Autologous Stem Cell Transplant, PLD- pegylated liposomal doxorubicin

Double-refractory = refractory to both bortezomib and lenalidomide

* Based on original 2013 mSMART risk.(24)

Table 2:

Efficacy of KDD in Patients Treated at the MTD

Overall Response Rate	83% (95% CI 0.68–0.98)
Best Overall Response	
CR/sCR	25%
VGPR	29%
PR	29%
SD/No Response	17%
Estimated Median PFS (months)	13.4 (95% CI 5.0–21.7)
Estimated Median OS (months)	Not Reached
1 Year OS	83%
Overall Response Rate in PI-refractory	60%
Overall Response Rate in PI-responsive/naïve	100%