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AMC-063: Safety and Efficacy of Bortezomib in AIDS-associated Kaposi Sarcoma

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Abstract

Purpose: AIDS-related Kaposi sarcoma (KS) is often incompletely controlled, requiring serial therapies. KS herpesvirus (KSHV) induces transformation of endothelial cells, where it resides in a predominately latent state. We hypothesized proteasome inhibition would have direct anti-tumor activity, induce lytic activation of KSHV, and inhibit HIV infectivity, improving control of both KS and HIV. The primary objective was determining the maximum tolerated dose (MTD) of

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Authorship Contributions:

EGR and WW led study design, implementation, analysis, and writing of the paper. EGR additionally managed patients enrolled in the study.

AS participated in patient management and contributed to the analysis and writing of the paper.

SL contributed to statistical methods and analysis as well as writing of the paper.

DPD conducted analysis of gene expression profiling, contributed to study design and writing of the paper.

RA contributed to study design, patient management, KSHV VL testing, contributed to study design and writing of the paper.

FM and RG conducted single copy HIV VL testing and writing of the paper; FM also contributed to study design.

DA and RM contributed to patient management, study design and writing of the paper.

MD contributed to patient management and writing of the paper.

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bortezomib in AIDS-KS. Secondary objectives included estimating the impact of bortezomib on KS response, KSHV plasma DNA copy number (PDCN) and HIV viral loads (VL).

Methods: A 3+3 dose escalation design was employed evaluating 4 dose levels of bortezomib (0.75, 1, 1.2, or 1.6 mg/m^2) administered intravenously on days 1, 8, and 15 of 28-day cycles in patients with relapsed/refractory (r/r) AIDS-KS taking antiretroviral therapy.

Results: Seventeen patients enrolled. No dose limiting toxicities occurred and the MTD was not reached. The most common adverse events included diarrhea, fatigue and nausea. Among 15 patients evaluable, partial response (PR) occurred in 9 (60%), with PR rate of 83% in the 1.6 mg/m^2 cohort; the remainder had stable disease (SD). Median time to response was 2.1 months. Median change in KSHV PDCN was significantly different between those with PR versus SD. During cycle 1, 7 of 11 evaluable patients had decreases in HIV VL.

Conclusion: Bortezomib is well-tolerated and active in AIDS-KS. The 60% PR rate is notable given the dose-finding nature of the study in a r/r population. Changes in KSHV PDCN and HIV VL trended as hypothesized.

Introduction

Persons living with HIV (PLWH) are several thousand times more likely to be diagnosed with Kaposi Sarcoma (KS) than their HIV seronegative counterparts, and KS is one of the most commonly diagnosed malignancies among PLWH.[1,2] While the introduction of highly active anti-retroviral therapy (HAART) has been associated with decreased incidence of and increased survival rates in KS, complete remission is rare and there is no curative therapy. Immune reconstitution through use of HAART is an important component of therapy, but is often insufficient, especially with advanced disease.[3,4] Local therapies such as radiation, cryotherapy, intralesional or topical therapies may be used to control very limited cutaneous disease. More extensive cutaneous or visceral disease requires systemic treatment, such as liposomal anthracyclines or paclitaxel which have overall response rates ranging from 40–90%.[3,5,6] Small studies of targeted therapies and immunomodulatory agents demonstrating activity in AIDS-KS include imatinib (n = 30, ORR 33%)[7], thalidomide (n= 17, ORR 47%)[8], and pomalidomide (n= 15, ORR 60%)[9].

KS-associated herpesvirus (KSHV, also known as human herpesvirus-8, HHV-8) is the primary etiologic factor associated with KS. The virus infects endothelial cells, resulting in transformation into spindle cells characteristic of KS.[10] Residing in a predominately latent state within KS, KSHV promotes tumor cell survival. The exact mechanisms of KSHV-driven oncogenesis are unknown and may involve any number of viral genes, such as the latency associated nuclear antigen (LANA), the viral cyclin (vCYC) and a protein called vFLIP, which inhibit apoptosis and autophagy, and activate nuclear factor kappa B (NF-kB)[11] as well as the viral micro RNAs. Acting together, KSHV reprograms both infected and uninfected cells within the KS lesion.[12]

Bortezomib (Velcade™, Millennium Pharmaceuticals, Inc., Cambridge, MA) is a proteasome inhibitor which was initially approved for treatment of multiple myeloma and has activity in other malignancies attributed at least in part to inhibition of NF-kB.[13,14–16] We hypothesized bortezomib would be active in KS either as a result of inhibition of

proteasome function (required for NF-kB) or as a result of KSHV lytic activation resulting in direct tumor killing by viral-mediated lysis. Anti-tumor activity of bortezomib against KS-endothelial cell models and PEL has been noted in preclinical studies.[17,18] Similarly, bortezomib-mediated viral lytic gene expression has been seen in cell-line xenograft studies.[19] Bortezomib has been identified as a potent lytic activator of Epstein Barr virus (EBV), a gammaherpesvirus similar to KSHV,[20,21] as well as KSHV in the context of the KSHV-associated primary effusion lymphoma (PEL).[22–24] Lytic activation of the latent KSHV infecting KS tumors may result not only in direct tumor cell lysis but also in increased antigen expression and immune recognition of KS, as lytic viral proteins are often more immunogenic than their latent counterparts. Alternatively, lytic activation may conceivably result in viral inflammatory syndromes such as multicentric Castleman's disease or KSHV-associated inflammatory cytokine syndrome (KICS), or even drive tumorigenesis.

In addition to the inhibitory effect of bortezomib on tumors latently infected by gammaherpesviruses, pre-clinical models suggest that bortezomib may suppress HIV via the cytidine deaminase, APOBEC3G, an innate cellular human defense against retroviruses. Human APOBEC3G is incorporated into nascent retroviral virions as they bud from the host cell and hypermutates viral cDNA within newly infected cells, thereby impairing faithful viral replication (Figure 1a). HIV evades this defense by employing Virion Infectivity Factor (Vif), which induces the ubiquitination of APOBEC3G and targets the protective protein for proteasomal degradation (Figure 1b). *In vitro* studies inhibiting human 26S proteasome have demonstrated impairment of HIV viral budding and infectivity in association with restoration of host APOBEC3G levels (Figure 1c).[25,26] We recently reported an increase in APOBEC3G levels in patients with HIV lymphoma treated with bortezomib,[27] suggesting bortezomib has the potential to contribute to suppression of HIV through restoration of this protective factor. Given the important role immune reconstitution plays in control of AIDS-KS, this additional anti-retroviral activity may be beneficial.

Patients and Methods:

Protocol design and therapy

A 3+3 dose escalation design of single-agent bortezomib was used to determine the maximum tolerated dose (MTD) of bortezomib and to evaluate the clinical response of KS tumors to bortezomib. Bortezomib was administered intravenously on days 1, 8, and 15 of each 28-day cycle at one of 4 dose levels: 0.75 mg/m^2, 1 mg/m^2, 1.2 mg/m^2, and 1.6 mg/m^2, respectively.

This study was conducted in accordance with the Declaration of Helsinki. Institutional review boards at each of the study sites approved the study protocol in accordance with an assurance filed with and approved by the U.S. Department of Health and Human Services. All patients provided written informed consent prior to study procedures and treatment.

Required supportive care included chemoprophylaxis against herpes zoster, pneumocystis jiroveci pneumonia (PJP) for patients with CD4+ counts <200 cells/mm^3, and Mycobacterium-avium complex (MAC) for patients with CD4+ counts <50 cells/mm^3. Granulocyte-Colony Stimulating Factor was not required for neutropenia prophylaxis, but

was permitted for treatment when clinically indicated. The dose limiting toxicity (DLT) period was defined as 2 cycles (8 weeks). Patients who tolerated bortezomib without experiencing any DLTs and had at least stable disease or better after 2 cycles were allowed to continue on protocol therapy for a maximum of 8 cycles. Intra-patient dose escalation to the next dose level was permitted in patients without DLTs after cycle 2.

Given the theoretical risk of inflammatory complications of lytic viral replication, patients were monitored clinically for development of life-threatening inflammatory syndromes (including Multicentric Castleman's disease, severe immune reconstitution syndrome or KSHV inflammatory cytokine syndrome); the occurrence of any such event would require discontinuation of protocol therapy. Monitoring for such inflammatory syndromes included regular clinical examination utilizing history and vital signs to evaluate for new onset fatigue, dyspnea, myalgias, arthralgias or fevers; physical exam to evaluate for progression of cutaneous KS, lymphadenopathy or development of effusions; and laboratory investigations to assess for cytopenias (complete blood count with differential), hyponatremia or organ failure (complete metabolic panel), or inflammation (c-reactive protein). Chest imaging was required at baseline and at onset of pulmonary symptoms to evaluate for progressive KS, development of effusions, or infectious complications.

Key Eligibility Criteria

Inclusion criteria included confirmation of HIV seropositivity with an FDA-approved and licensed test. KS diagnosis had to be confirmed by biopsy and relapsed after or refractory to at least one other prior frontline systemic therapy, either liposomal doxorubicin or paclitaxel. Individuals must have had at least five measurable cutaneous KS lesions suitable for assessment in addition to lesions for baseline and on study biopsies. Patients were required to be on a stable (≥12 weeks) PI-based or NNRTI-based ART regimen consisting of at least three drugs, with no intention of changing the regimen once on study. Other key inclusion criteria included: age ≥18 years, absolute neutrophil count ≥ 1000/mm^3, hemoglobin ≥ 8.0g/dL, platelet count ≥100,000/mm^3, total bilirubin ≤1.5mg/dL, AST/ALT ≤ 2x ULN, and creatinine clearance ≥ 50mL/min/1.73m^2 as calculated by the Cockcroft-Gault equation. Patients were excluded if they had any improvement of KS during the 4 weeks prior to enrollment, or if they had symptomatic visceral or pulmonary KS. Patients with oral and/or lymph node involvement, and/or asymptomatic pulmonary KS not interfering with activities of daily living were eligible. Other key exclusion criteria included an Eastern Cooperative Oncology Group performance score >2, grade >2 baseline peripheral neuropathy, concurrent active opportunistic infection, concurrent additional malignancy, serious medical illness requiring acute therapy within 14 days prior to study entry, hypersensitivity to Boron, or expected survival < 3 months.

Measurements and Assays

AIDS Clinical Trials Group (ACTG) KS Staging Classification was implemented to determine staging of disease at study entry. Two 3-mm punch biopsies were collected from a KS lesion at baseline, cycle 1 day 2, and at treatment discontinuation. One of the two biopsies was submerged in RNAlater for RNA isolation, while the other was fixed in formalin for further assessment by immunohistochemistry (IHC).

IHC from biopsy specimens was performed to evaluate tumor content and proliferation (by Ki-67), and presence of KSHV (LANA). Messenger RNA was isolated from RNA-later preserved biopsies using real-time reverse-transcription quantitative polymerase chain reaction (RT-qPCR) to measure the levels of all KSHV genes in the tumor, as described.[28]

Blood samples were collected at baseline, Cycle 1 Day 1, 2, 4, 8, 15 and Cycle 2 Day 1. KSHV PCR on DNA extracted from plasma was carried out as described previously.[29] Plasma HIV viral load was determined using RT-qPCR, as previously described.[30] HIV plasma samples were also collected on day 1 of subsequent cycles prior to bortezomib administration.

Tumor response assessments were performed at baseline, weeks 4, 8, 12, 16, and 20, and then every 3 months for up to one year using response definitions outlined in the protocol (see Supplemental Methods). The Functional Assessment of Cancer Therapy/Gynecologic Oncology Group–Neurotoxicity (FACT-GOG/NTX) questionnaire[31] was administered at baseline, Day 15 of cycle 1 and the first cycle of intra-patient dose escalation, and on the fourth week of every subsequent treatment cycle.

Statistics

Descriptive statistics were reported for demographic data, baseline disease characteristics, adverse events, and KS response rates. Binomial proportions with 95% confidence intervals were calculated to estimate the overall KS response rates for all patients considered evaluable for response. The Wilcoxon signed rank test was used to investigate the changes from pre- to post-bortezomib administration on HIV viral loads from serum, on KSHV copy number from PBMC, plasma, and FACT-GOG/NTX scores. Unsupervised clustering and principal component analysis were applied to explore KSHV transcription in KS lesions.

Results

Seventeen patients were enrolled across six different sites within the AIDS Malignancy Consortium (AMC). As shown in Table 1, all of the patients were males with a median age (range) of 45 (29–59) years. At baseline, 59% (n=10) of patients had disease involvement confined to the skin (without associated edema or ulcerations) and/or to lymph nodes and/or had minimal oral disease. All but 2 of the 17 patients had a CD4+ count >200 cell/uL (88%, n=15). No patient had active organ or visceral involvement with KS. Fourteen patients (82%) had a Karnofsky Performance Status (KPS) of >70. Thirteen patients (76%) had an undetectable HIV viral load at baseline.

Three patients were enrolled into each of the four dose escalation cohorts of the study without occurrence of a DLT. The MTD was not reached at the highest dose, 1.6 mg/m^2, which was selected for a dose expansion cohort to which an additional 3 patients were enrolled. Of the 3 patients, two withdrew early from the study prior to completing two cycles of therapy so were not evaluable for response and were replaced. The first patient declined continued protocol therapy and follow-up, and the second withdrew from the trial due to scheduling difficulties. A total of 6 patients treated at the highest dose level were therefore

evaluable for response. One patient in cohort 1 and 2 patients in cohort 2 dose-escalated to the next higher dose level for their final 2 cycles of protocol therapy.

Of the 17 patients enrolled and treated on study therapy, all of them experienced at least one adverse event (AE). There were a total of 177 AEs experienced, the most common of which were diarrhea (53%), fatigue (53%) and nausea (41%). Nearly two-thirds of all AEs (n=112/177) were attributed as at least possibly related to bortezomib, and were experienced by 15 (88%) of participants (Table 2).

Three patients experienced serious adverse events (SAE). One patient had severe vomiting. A second patient had two separate infectious SAEs, both occurring without associated neutropenia or lymphopenia: bacteremia assessed as grade 4 sepsis in the context of sinusitis and pneumonic process, and a grade 3 cellulitis of the thigh at site of KS. A third patient experienced fatal cardiac arrest in the context of endocarditis without associated neutropenia or lymphopenia; his endocarditis was attributed to a PICC line infection occurring in the context of performing gardening and heavy landscaping work without adequate protection of the PICC line from exposure.

Grade 1 peripheral neuropathy was reported in 4 patients (24%), and an additional patient developed grade 2 neuralgia. Neurotoxicity as measured by the FACT/GOG-Neurotoxicity Questionnaire did not significantly increase at any assessment point in this small study. With a possible score range of 0–44, the median baseline score was 6. The median change from baseline was −0.5 (interquartile range (IQR),−1.5 to 6.0) by treatment discontinuation (n=12, p=0.402). The maximum score increase was 10 points, occurring in 2 patients, corresponding to development of grade 1 peripheral sensory neuropathy.

Of 303 planned doses, 89% were administered on time at the protocol-specified dose. There were 21 dosing delays of which 11 (52%) were due to an adverse event and others were due to patient preference or scheduling. The median delay was 7 days. There were 4 dose reductions, 2 of which were due to adverse events, 1 due to physician error and the other due to patient request. Two patients discontinued due to adverse events at least possibly related to bortezomib (grade 3 neutropenia considered definitely related but would not have required protocol discontinuation, and grade 2 lymphedema considered possibly related).

Among the 15 patients that were evaluable for response, 60% (95% CI, 32–84%) experienced a partial response (PR) as their best response to therapy, whereas the remaining patients had stable disease (SD) (Table 3). Five of 6 patients treated at the highest dose level experienced a PR (83%). The median time to response was 2.1 (IQR, 1.9 to 2.8) months. Examples of lesion improvement as early as post cycle 2 are included in Supplemental Materials. By the end of the study, none of the patients who experienced a PR progressed after a median of 2.8 (IQR, 1.9 to 18.5) months from initial response. There was no disease progression while on protocol therapy. One patient with SD progressed 446 days after enrollment.

Median change in plasma KSHV DNA copy number during cycle 1 compared with baseline was negligible in patients obtaining partial remission. In contrast, the median plasma KSHV DNA copy number decreased compared with baseline in patients with stable disease. The

difference between responders and non-responders was statistically significant after the first treatment on both cycle 1 day 2 and Day 8 (Figure 2). In the cohort treated at the highest dose level of bortezomib, 7 of 8 patients were evaluable for changes in KSHV plasma DNA copy number during cycle 1. Of these 7, 6 patients had increases (range of maximum increase: 71 – 40,517 copies/ml) while the other patient's KSHV was persistently undetectable. The timing of the maximum increase in KSHV copy number during the first cycle was variable among patients.

The numbers of tumor cells in KS lesions as assessed by immunohistochemistry and mRNA profiling varied widely among patients. Figure 3 shows a heat map depiction of relative mRNA levels for KSHV and indicator endothelial cell (EC) mRNAs. Darker Red colors indicate more and lighter yellow colors less transcription. The data were adjusted for equal mRNA levels using a combination of multiple "housekeeping" genes. The mRNAs clustered into three broad categories: "EC", which is comprised of human EC markers, "latent", which is comprised of KSHV latent mRNAs, that should be present in every infected cell, and "lytic", which is comprised of KSHV mRNAs that are associated with lytic reactivation in PEL cell lines. The biopsies clustered into three clusters "a", "b", and "c". Cluster "c" represents lesions with high level KSHV activity, cluster "b" represents lesions with a large proportion of EC but KSHV was present in only a minority of EC and predominantly in a latent state. Finally, cluster "b" represents lesions with virtually no endothelial cells and no KSHV, i.e. fibrotic tissue rather than tumor tissue. The immunohistochemistry analyses confirm the profiling data. Every biopsy stained positive for Ki-67, had clusters of KSHV LANA-positive spindle cells, but often these clusters consisted of very few cells.

Of 11 patients evaluable for change in HIV VL from baseline during cycle 1, 7 had a decrease (range of maximum absolute decrease: 2.5–1,330 copies/ml), and 4 had an increase in HIV VL (range of maximum absolute increase: 0.8 to 54 copies/ml). Of the 5 patients with >1 log decrease in HIV VL during cycle 1, 4 experienced PR and the other had SD.

Discussion

Administration of bortezomib was safe and well-tolerated, with only mild-moderate adverse effects attributed to bortezomib, and showed excellent activity in AIDS-KS with 100% disease control rate. No DLTs occurred at the highest tested dose level at which all but 1 patient experienced a partial response. Recognizing this is a phase I study with small numbers, comparisons to other therapies are inherently limited. However, bortezomib demonstrated a high proportion of responses in a population refractory to standard frontline therapy of KS, with few cytopenias, a low proportion of dose reductions or delayed treatment, and lack of alopecia.

Increased risk of infections and neuropathy are known complications of both bortezomib and HIV infection. Severe infections were observed in the trial and assessed as unlikely related to study therapy due to alternate attributions. However, given the well-documented increased risk of infections due to proteasome inhibitor treatment in other disease contexts, vigilant monitoring remains important when using proteasome inhibition in this population. Neurotoxicity was not found to be a limiting adverse event of this bortezomib dosing

scheme in AIDS-KS. As intravenous administration of bortezomib was standard of care at the time of protocol design, subcutaneous administration was not employed, but would be expected to further reduce risk of neuropathy.[32]

Changes in HIV viral loads were not statistically significant, however, there was a trend toward decreased HIV viral loads during cycle 1 as predicted. The small size of the study and undetectable baseline HIV viral loads in 76% of patients limited sensitivity and interpretation of the data despite single copy viral load testing.

KSHV copy number in plasma varied considerably from patient to patient. In general, viral DNA persisted in plasma throughout the course of the study. There was a trend toward increased KSHV plasma DNA in patients receiving the highest dose of bortezomib (1.6 mg/m^2), an expected finding with hypothesized lytic KSHV activation. However, it is not clear whether this reflected infectious virion production as might be expected from a lytic activator, or merely the release of viral DNA from dying KS cells. We note that no inflammatory syndromes were observed in the trial, a potential risk of viral lytic activation for which the trial specified monitoring. No patient with stable disease had increase in KSHV copy number during cycle 1 and the median plasma KSHV DNA copy number decreased compared with baseline in patients with stable disease. The explanation for this trend in non-responding patients is under investigation.

Mechanistic and molecular studies on tumor tissue were difficult to interpret due to the small number of KS spindle cells in the biopsies at baseline. The presence of two types of KS differing by the degree of KS transcription recapitulates prior studies of KS from ART-naive patients in Sub-Saharan Africa and AIDS-KS patients in the US.[28,33] The fact that over half of the biopsies exhibited minimal EC and KS transcription may reflect the patient composition of AMC-063, which enrolled only patients on successful ART in the US. Multiple factors limited sensitivity to changes in KSHV viral gene expression: on-treatment biopsies were limited to a single assessment performed only 24 hours after initial bortezomib exposure, not all baseline biopsies had robust evidence of KSHV transcription, and we were not able to biopsy the same lesion multiple times.

Bortezomib has multiple, divergent effects in KSHV-associated malignancies. The bulk of mechanistic studies have been conducted in the KSHV-associated primary effusion lymphoma, which is dependent on NFkB signaling.[34] Investigations in KSHV-driven endothelial cell models are more limited.[17] The molecular mechanisms and specific targets underlying the efficacy of bortezomib in KS are still unknown. The most likely scenario includes both direct actions of bortezomib on cellular pathways independent of KSHV, perhaps through induction of an unfolded protein response, as well as actions of bortezomib on KSHV reactivation from latency, which lead to cell death.

Reactivating KSHV from latency comes with a theoretical risk as KSHV lytic proteins can have pro-tumorigenic, pro-inflammatory and immune suppressive phenotypes[35] and as lytic replication may foster further dissemination of the virus. Inducers of KSHV reactivation without direct cellular toxicity have demonstrated limited efficacy in KS.[36] The combination of direct cellular cytotoxicity and potential to reactivate KSHV found in bortezomib may

have contributed to the clinical efficacy of bortezomib and did not result in KSHV-associated inflammatory syndromes. The study is somewhat limited given the small sample size and relatively short median follow-up. Although prophylaxis with valganciclovir to protect against KSHV activation has been suggested, its use is controversial, and in the patients treated in this trial without such prophylaxis, no adverse effects attributable to KSHV viremia were identified. Furthermore, there were no cases of tumor progression during bortezomib therapy.[37,38]

While the precise mechanism of action of bortezomib as an anti-tumor agent in KS remains unclear, the data presented demonstrate that bortezomib is active in patients who have failed standard therapies. Despite concerns about potential risks of lytic KSHV activation, bortezomib was well tolerated and neither tumor progression nor other indications of adverse events attributable to viral lytic activation were seen. Therefore, further evaluation of proteasome inhibition is warranted. Given that the highest burden of KS falls upon resource-limited countries, evaluation of strategies employing an orally administered proteasome inhibitor or combination therapies including proteasome inhibitors in KS would be attractive. Based on the encouraging results of this study, the AIDS Malignancy Consortium is developing a trial using an oral proteasome inhibitor in Kaposi sarcoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Translational Relevance

This trial is the first to demonstrate clinical activity of proteasome inhibition in the management of Kaposi sarcoma (KS). Administration of bortezomib was safe with only mild-moderate adverse effects and showed excellent activity in AIDS-related KS with 100% disease control rate and partial responses occurring in 5 out of 6 patients treated at the highest dose level. Compared to other currently utilized treatments for relapsed/refractory AIDS-related KS, bortezomib demonstrated an equivalent or greater response rate, with few required dose reductions or delays and lack of alopecia or skin toxicities. Despite concerns about potential risks of lytic KSHV activation, bortezomib was well-tolerated and neither tumor progression nor other indications of adverse events attributable to viral lytic activation were seen. Given that the highest burden of KS falls upon resource-limited countries, evaluation of strategies employing orally administered proteasome inhibitors or combination therapies including proteasome inhibitors in KS would be attractive.

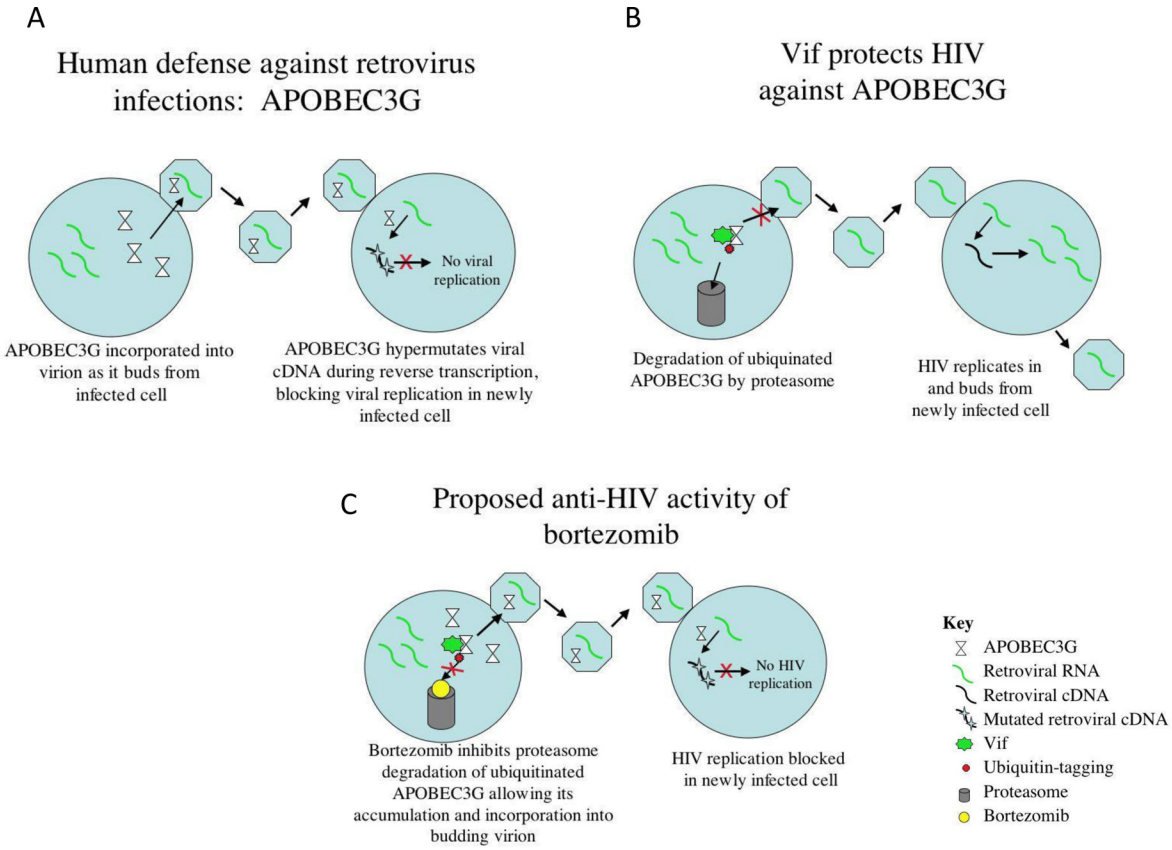

Figure 1. Mechanism of hypothesized anti-retroviral effects of bortezomib.

Humans have an innate defense mechanism against retroviral infections employing a cytidine deaminase, APOBEC3G. A. APOBEC3G is incorporated into nascent retroviral virions as they bud from an infected cell. As the nascent virion infects a cell and begins transcribing and copying its genes into the cellular DNA, APOBEC3G hypermutates the viral DNA to a point where replication is impossible, breaking the chain of infection. B. HIV circumvents this antiviral pathway using one of its accessory genes, virion infectivity factor (VIF). VIF induces ubiquitinization of APOBEC3G, thereby targeting this factor for proteasome degradation. In this way, VIF effectively shortens the half-life of APOBEC3G within the cell and prevents its incorporation into new virions. C. Proteasome inhibition would be expected to reverse the effects of VIF by slowing the degradation of APOBEC3G allowing it to be incorporated into budding HIV virions, impairing faithful viral replication.

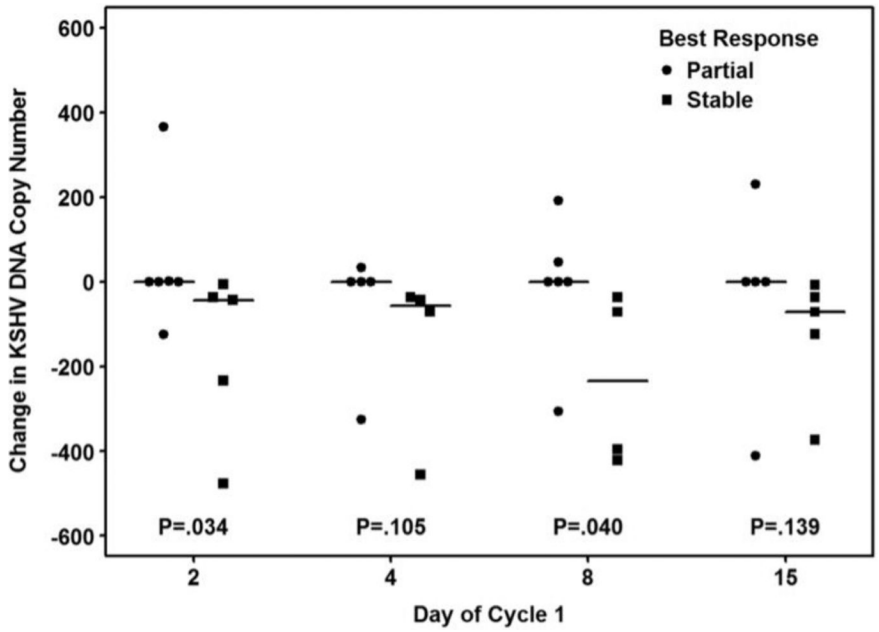

Figure 2. Change in KSHV DNA Copy Number from Baseline for Cycle 1 according to Best Response.

Change in plasma KSHV DNA copy number during cycle 1 was compared with baseline. The median change (represented by horizontal lines) was negligible in patients obtaining partial remission (individual patient values represented as dots). In contrast, the median plasma KSHV DNA copy number decreased compared with baseline in patients with stable disease (individual 37 patient values represented as squares). The difference between responders and non-responders was statistically significant after the first treatment on both cycle 1 day 2 and Day 8.

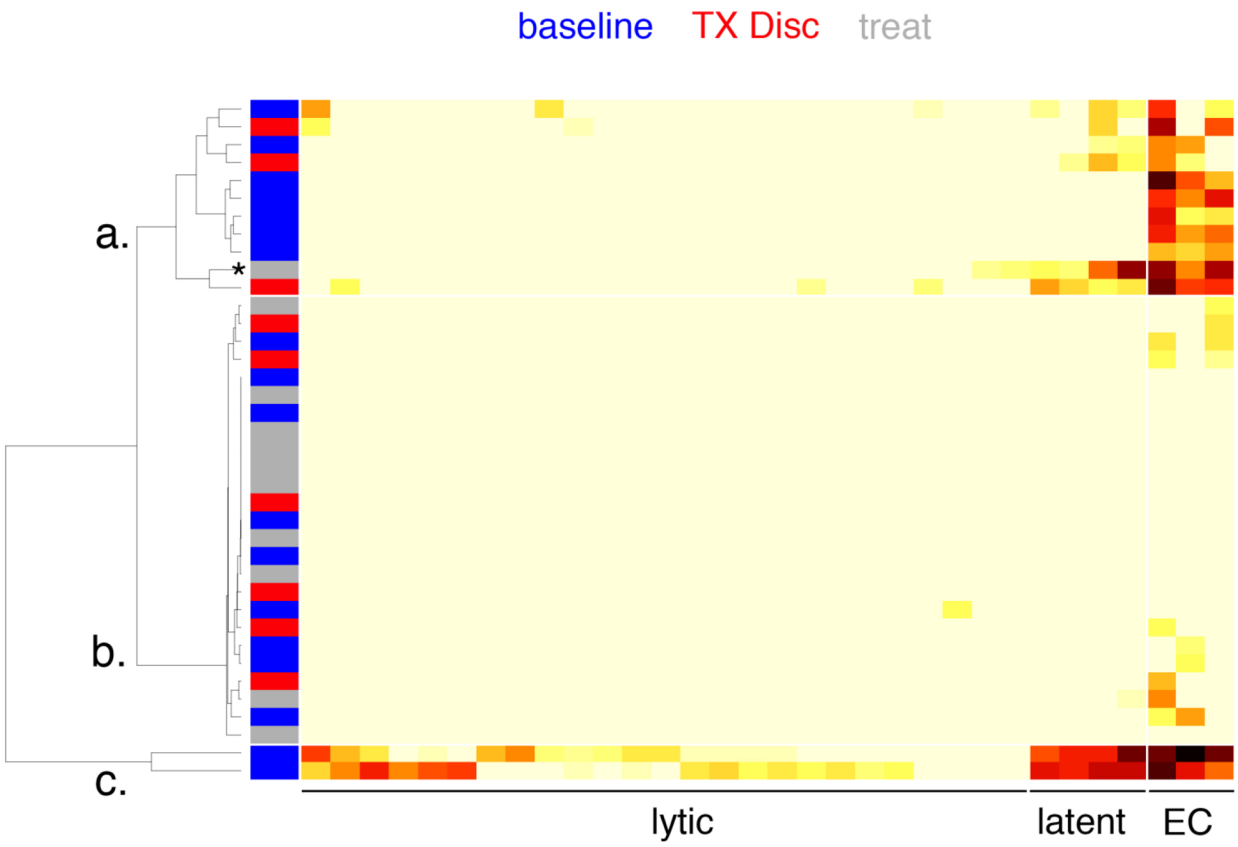

Figure 3. Relative mRNA levels for KSHV and indicator endothelial cell mRNAs

Shown is a heat map depiction of relative mRNA levels for KSHV and indicator endothelial cell (EC) mRNAs. Darker Red colors indicate more and lighter yellow colors less transcription. Clusters of samples are indicated by letters “a”, “b”, and “c”. 1 /11 (10%) of biopsies taken day 2 of cycle 1 (gray) had evidence of KSHV transcription (asterisk), nine (90%) biopsies taken day 2 of cycle 1 showed no molecular evidence of KSHV or EC (cluster “b”). 9/18 (50%) of baseline biopsies and 3/9 (33%) biopsies take at treatment discontinuation were classified as KSHV/EC transcription positive.

Table 1:

Patient Characteristics (n=17)

Demographics	
Age *years*, median (range)	45 (29–59)
Gender, n (%)	
Male	17 (100)
Race/Ethnicity, n (%)	
Non-Hispanic, White	8 (47)
Non-Hispanic, Black/AA	3 (18)
Non-Hispanic, Multiracial	2 (12)
Hispanic, White	3 (18)
Unknown/Not Reported	1 (6)
Staging at Registration	
Tumor Stage, n (%)	
1- Tumor is confined to skin and/or to lymph nodes and/or patient has minimal oral disease	10 (59)
2- Tumor with edema, ulceration, or extensive oral KS or gastro KS or KS in other non-nodal viscera	7 (41)
Immune System Staging, n (%)	
CD4 Cells ≥ 200/μL	15 (88)
CD4 Cells < 200/μL	2 (12)
History of Thrush/Opportunistic Infection, n (%)	
Yes	7 (41)
No	10 (59)
Karnofsky Performance Status, n (%)	
< 70	3 (18)
≥ 70	14 (82)
HIV Viral Load, n (%)	
Detectable	4 (24)
Undetectable	13 (76)

Table 2:

Adverse Events. Total adverse events occurrences are presented by category and grade followed in parentheses by the number of occurrences attributed as at least possibly related to bortezomib.

Organ System / Adverse Event		Severity Grade Total Number of Occurrences (Number attributed as at least possibly related to bortezomib)					
		Mild	Moderate	Severe	Life-threatening	Death	TOTAL
Blood And Lymphatic System Disorders	Anemia	3 (3)		1(1)			4 (4)
Cardiac Disorders	Cardiac Arrest					1	1
	Sinus Tachycardia		1(1)				1(1)
Ear And Labyrinth Disorders	Hearing Impaired	1					1
Eye Disorders	Blurred Vision	1	2				3
	Conjunctivitis	1					1
Gastrointestinal Disorders	Abdominal Pain	1(1)	1(1)				2(2)
	Constipation	2(2)					2(2)
	Diarrhea	7(5)	6(5)				13(10)
	Dry Mouth	1(1)					1(1)
	Gastroesophageal Reflux Disease		2(2)				2(2)
	Mucositis Oral	1(1)					1(1)
	Nausea	5(5)	6(5)	1(1)			12(11)
	Vomiting	4(3)	2(2)	1(1)			7(6)
General Disorders And Administration Site Conditions	Chills	4(2)					4(2)
	Edema Limbs	1(1)	1				2(1)
	Fatigue	8(7)	2(2)				10(9)
	Fever	5(4)	1(1)				6(5)
	Malaise	1(1)					1(1)
	Non-Cardiac Chest Pain	1	1				2
	Pain		4				4
Infections And Infestations	Endocarditis Infective			1			1
	Infections And Infestations - Other, Specify		1				1

Organ System / Adverse Event		Severity Grade Total Number of Occurrences (Number attributed as at least possibly related to bortezomib)					
		Mild	Moderate	Severe	Life-threatening	Death	TOTAL
	Sepsis				1		1
	Sinusitis		3				3
	Skin Infection		2(1)	2	1		5(1)
	Upper Respiratory Infection		2(1)				2(1)
Investigations	Alanine Aminotransferase Increased	1(1)					1(1)
	Blood Bilirubin Increased			1			1
	Cholesterol High		1(1)				1(1)
	Lymphocyte Count Decreased		1(1)				1(1)
	Neutrophil Count Decreased		2(2)	3(3)			5(5)
	Platelet Count Decreased	4(3)					4(3)
	White Blood Cell Decreased	5(5)	1(1)				6(6)
Metabolism And Nutrition Disorders	Hyperglycemia	3					3
	Hyperkalemia		1				1
	Hypocalcemia	5(5)					5(5)
	Hyponatremia	7(6)		1			8(6)
Musculoskeletal And Connective Tissue Disorders	Arthralgia		1				1
	Back Pain	1(1)	2	1			4(1)
	Muscle Weakness Lower Limb	1(1)					1(1)
	Myalgia	1(1)					1(1)
	Pain In Extremity		3	1			4
Nervous System Disorders	Dizziness	2(2)					2(2)
	Headache	5(4)	2(1)				7(5)
	Memory Impairment	2					2
	Nervous System Disorders - Other	2(1)					2(1)
	Peripheral Motor Neuropathy	2(1)					2(1)
	Peripheral Sensory Neuropathy	2(1)	1(1)				3(2)
	Tremor			1			1

Organ System / Adverse Event		Severity Grade Total Number of Occurrences (Number attributed as at least possibly related to bortezomib)					
		Mild	Moderate	Severe	Life-threatening	Death	TOTAL
Psychiatric Disorders	Anxiety		1(1)				1(1)
	Depression		3				3
Renal And Urinary Disorders	Acute Kidney Injury	1(1)					1(1)
Respiratory, Thoracic And Mediastinal Disorders	Cough	1(1)	1(1)				2(2)
	Dyspnea	1	1				2
	Pneumonitis		1(1)				1(1)
	Sinus Disorder	1(1)					1(1)
Skin And Subcutaneous Tissue Disorders	Hyperhidrosis	1(1)					1(1)
	Skin And Subcutaneous Tissue Disorders - Other		2				2
Vascular Disorders	Hypertension		1				1
	Lymphedema		2(2)				2(2)
	Superficial Thrombophlebitis		1				1
TOTAL (Occurrences)		95(72)	65(34)	14(6)	2	1	177(112)

Table 3:

Best Response*

	Bortezomib 0.75 mg/m^2 N=3	**Bortezomib 1.0 mg/m^2 N=3**	**Bortezomib1.3 mg/m^2 N=3**	**Bortezomib 1.6 mg/m^2 N=6**	**All Doses N=15**
Partial Response, n (%)	1 (33)	3 (100)	0 (0)	5 (83)	9 (60)
Stable Disease, n (%)	2 (67)	0 (0)	3 (100)	1 (17)	6 (40)

*Note: Two patients (not shown above) were not evaluable for response as they did not complete 2 cycles of therapy. Intra-patient dose escalation to the next dose level was permitted in patients without DLTs after cycle 2. One patient in cohort 1 and 2 patients in cohort 2 dose-escalated to the next higher dose level for their final 2 cycles of protocol therapy.