Cutting Edge

THE JOURNAL OF IMMUNOLOGY

Cutting Edge: Bortezomib-Treated Tumors Sensitized to NK Cell Apoptosis Paradoxically Acquire Resistance to Antigen-Specific T Cells

Andreas Lundqvist,[1] Su Su, Sheila Rao, and Richard Childs

Bortezomib augments caspase-8 activity, rendering tumors susceptible to NK cell lysis. We hypothesized this effect would likewise sensitize tumors to Ag-specific CTLs. Instead, bortezomib-treated tumors that acquired sensitivity to NK cells simultaneously became resistant to killing by Ag-specific CTLs. Reduction in CTL killing persisted for days, was not due to changes in tumor expression of MHC class I, and was overcome by pulsing tumors with peptides recognized by tumor-reactive CTLs. Tumor-outgrowth experiments showed tumors grew faster in SCID mice when cocultures of tumor-reactive CTLs and bortezomib-treated tumors were injected compared with untreated tumors (tumor doubling time 3.1 and 10.6 d, respectively; $p < 0.01$), whereas tumors grew slower in mice receiving cocultures of NK cells and bortezomib-treated tumors compared with untreated tumors (11.8 d and 5.0 d, respectively; $p < 0.01$). These findings demonstrate bortezomib-treated tumors sensitized to NK cell apoptosis paradoxically acquire resistance to CTLs as a consequence of bortezomib altering proteasomal processing and presentation of tumor Ags. *The Journal of Immunology*, **2010, 184: 1139–1142.**

Recently, researchers have explored novel methods to overcome tumor resistance to the apoptotic effects of immune effector cells as an adjunct to cancer immunotherapy (1, 2). A number of anticancer agents including bortezomib and romidepsin have recently been shown to sensitize tumors to death receptor signaling pathways used by both NK cells and T cells to induce tumor apoptosis (3–6). We and others have demonstrated that both human and murine tumors exposed to bortezomib upregulate TRAIL death receptors and have augmented caspase-8 activity, which enhances their susceptibility to NK cell killing via TRAIL, Fas ligand, and perforin granzyme (5, 7–9). Because CTLs also induce tumor apoptosis via these pathways, we hypothesized that bortezomib would likewise sensitize tumors to Ag-specific CTLs, thereby serving as a sensitizing agent to both innate and adaptive cellular immunity.

In this study, we demonstrate exposure of cells to bortezomib simultaneously results in divergent effects on NK cell and T cell antitumor immunity, whereas bortezomib-treated tumors became sensitized to NK cell apoptosis and drug-induced proteasome inhibition altered tumor-Ag presentation, paradoxically reducing tumor-specific T cell effector responses.

Materials and Methods

Cells and reagents

NK cells, melanoma-reactive JKF6 and L2D8 (melanoma Ag recognized by *T cells* /MART-1]: 26–35/A2 and gp100: 209–217/A2), $CD8^+$ T cell clones, minor histocompatibility Ag, and EBV-specific T cells were generated using described methods (5, 10, 11). The melanoma cell lines 526mel and 624mel (HLA-$A2^+$/$gp100^+$/MART-1^+), 938mel and 888mel (HLA-$A2^-$/$gp100^+$/MART-1^+), and K562 and T2 cell lines were maintained in RPMI 1640 (Cellgro, Herndon, VA) with 10% human FCS (HyClone, Logan, UT). EBV-lymphoblastoid cell lines and B cells were generated as previously described (11). Flow cytometric assays (CD3, CD16, CD56, MIC-A/B, HLA-ABC; BD Pharmingen, Franklin Lakes, NJ), anti-Fas (ZB4; Abcam, Cambridge, MA), and DR4 and DR5 (Biolegend, San Diego, CA) were acquired on FACSCalibur (BD Pharmingen). Bortezomib (Millennium Pharmaceuticals, Cambridge, MA) and romidepsin (FK228, depsipeptide; Fujisawa Pharmaceuticals, Osaka, Japan) were purchased from the National Institutes of Health Division of Veterinary Resources pharmacy. Intracellular cytokine staining for IFN-γ was performed according to the manufacturer's instructions (BD Pharmingen).

Apoptosis, cytotoxicity, and proliferation assays

Apoptosis was measured by Annexin V (BD Pharmingen) and 7-aminoactinomycin D (Beckman Coulter, Fullerton, CA) staining. [^{51}Cr] (PerkinElmer, Wellesley, MA) cytotoxicity assays were performed by plating [^{51}Cr]-labeled target cells with NK or T cells for 4 h as described previously (6). T cell proliferation was analyzed by thymidine uptake. Tritiated thymidine (1 μCi/well) was added after 3 d and incubated for an additional 16 h before harvesting and analyzed as previously described (6). L2D8 cells were stained with CD3-PE and coculture with GFP-positive 526 melanoma (526mel) cells for 5, 30, 60, and 960 min and thereafter fixed and analyzed for formation of conjugates by ImageStream (Amnis, Seattle, WA).

Tumor outgrowth experiment

Luciferase-transduced 526mel cells were cocultured in vitro with allogeneic NK or L2D8 cells for 6 h and injected i.p into CB-17 SCID beige mice (Taconic, Rockville, MD). Tumor burden and tumor doubling time were calculated by injecting mice i.p with 2 mg/mouse D-luciferin (Caliper Life Sciences, Hopkinton, MA) every 3–7 d between days 9 and 44 following tumor injection and were imaged using the IVIS Xenogen system (Caliper Life Sciences). All experiments were approved by the National Heart, Lung, and

Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Bethesda, MD 20892

[1]Current address: Department of Oncology and Pathology, Cancer Center Karolinska, Karolinska Institutet, Stockholm, Sweden.

Received for publication August 28, 2009. Accepted for publication November 16, 2009.

This work was supported by the intramural research program of the National Institutes of Health, National Heart, Lung, and Blood Institute, Hematology Branch, and the Swedish and European Research Councils (to A.L.).

Address correspondence and reprint requests to Dr. Richard Childs, Hematology Branch, National Heart, Lung, and Blood Institute, National Institutes of Health, Room 3-5140, Building 10-CRC, 10 Center Drive, MSC 1202, Bethesda, MD 20892-1202. E-mail address: childsr@nhlbi.nih.gov

Abbreviations used in this paper: DC, dendritic cell; GVHD, graft-versus-host disease; GVT, graft-versus-tumor; p/s, photons per second.

www.jimmunol.org/cgi/doi/10.4049/jimmunol.0902856

Blood Institute animal care and use committee (Protocol H-0111R1). A $p < 0.05$ using the Student t test or Fisher's exact test was considered a significant difference between treatment groups.

Results and Discussion

The melanoma cell line 624mel was treated with 20 nM of bortezomib for 18 h and then tested for sensitivity to lysis by allogeneic NK cells and gp100-specific (L2D8) and MART-1–specific (JKF6) T cells. Bortezomib treatment alone did not significantly reduce tumor viability (Fig. 1*A*). Although NK cell cytotoxicity was increased against bortezomib-treated tumors, T cell cytotoxicity decreased against the same bortezomib-treated tumors compared with nontreated control tumors (Fig. 1*B*, 1*C*). Similarly, proliferation (Fig. 1*D*) decreased dramatically when L2D8 T cells were cocultured with bortezomib-treated 526mel cells compared with untreated tumors. Although a transient increased sensitivity to killing by NK cells was observed, T cell recognition of drug-exposed tumors was reduced for multiple days, not returning to baseline until 4 d after bortezomib was washed from tumors (Fig. 2). In most experiments, treatment of tumors with bortezomib decreased T cell-mediated tumor cytotoxicity substantially. Nevertheless, tumor killing never reached 0%, perhaps because the 20 nM dose of bortezomib was insufficient to completely inhibit the proteasome, allowing for some Ag presentation to persist. In some experiments, a further reduction of cytotoxicity was observed when the dose of bortezomib was increased up to 1000 nM (data not shown). Bortezomib also reduced the susceptibility of target cells to recognition and killing by viral Ag and minor histocompatibility Ag-specific T cells; both IFN-γ production and cytotoxicity (data not shown) by EBV-reactive and minor histocompatibility-reactive T cells was reduced when EBV-lymphoblastoid cell line targets were exposed to bortezomib compared with untreated controls (Fig. 3).

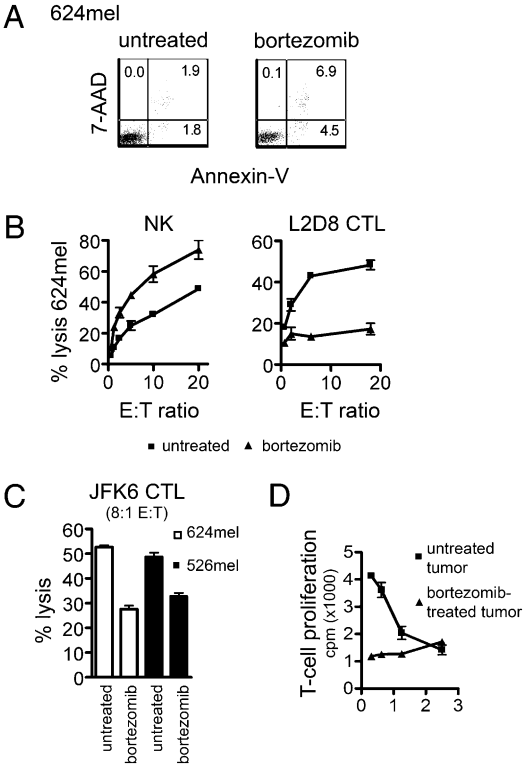

FIGURE 1. Tumors treated with bortezomib are sensitized to NK cell lysis while simultaneously acquiring resistance to Ag-specific T cells. *A*, Untreated or bortezomib-treated (20 nM, 18 h) melanoma cells (624mel/HLA-A*02 positive) were analyzed for apoptosis by annexin-V and 7AAD staining. *x*- and *y*-axis show log fluorescence. Similar results were obtained with the 526mel cell line (data not shown). Higher concentrations of bortezomib (up to 320 nM) did not induce significant apoptosis in these tumors (data not shown). Bortezomib-treated (20 nM, 18 h) 624mel or 526mel tumor cells were tested for susceptibility to lysis by allogeneic human NK cells, gp100-specific $CD8^+$ T cells (L2D8, directed against a gp100-derived peptide 209–217 in complex with HLA-A*02) (*B*) and MART-1–specific $CD8^+$ T cells (JKF6, directed against MART-1–derived peptide 27–35 in complex with HLA-A*02) in a 4 h [^{51}Cr] assay (*C*). JKF6 T cells were tested against tumor cells at an 8:1 E:T ratio. The tumor cell lines 938mel and 888mel (both HLA-A*02 negative) and K562 were not killed by either L2D8 or JKF6 cells (data not shown). *D*, L2D8 cells plated at 50,000 cells/well in a 96-well plate were tested for proliferation (3 d) in response to coculture with irradiated (100 Gy) 526mel cells that had been treated or not treated with bortezomib (20 nM, 18 h) at responder:stimulator ratios ranging from 0.3–5:1 in the presence of 10 U/ml of IL-2. cpm values for 526mel alone and responders alone were <100 and 800, respectively.

Using bioluminescent imaging, tumor outgrowth assays in SCID mice showed tumors grew significantly slower in mice receiving cocultures of bortezomib-treated human tumors and NK cells compared with recipients of untreated tumor/NK cell cocultures or bortezomib-treated tumors alone. In contrast, tumor growth was accelerated in mice receiving cocultures of bortezomib-treated human tumors and tumor-reactive T cells compared with mice receiving untreated tumor/T cell cocultures (Fig. 4). The reduction in T cell recognition of bortezomib-treated tumors did not occur as a consequence of bortezomib reducing tumor MHC class I expression; FACS analysis showed bortezomib-treated versus untreated tumors had similar surface expression of MHC class I, DR4, MIC-A/B, and Fas, although bortezomib significantly upregulated tumor expression of DR5 (5) (Fig. 5*A*). Prior studies have shown that bortezomib can indirectly reduce T cell alloreactivity by inhibiting dendritic cell (DC) maturation, phagocytosis, and IL-12 production and can directly suppress allogeneic T cell proliferation and Th1 responses (12–17). In contrast, the reduction in T cell immunity against tumor cells observed in our studies occurred as a direct effect of bortezomib on tumor cells because tumors were extensively washed precluding bortezomib-induced suppression of effector cells. Furthermore, in vitro studies showed reduced gp100-specific T cell cytotoxicity and IFN-γ production could be restored to baseline by culturing bortezomib-treated tumors with exogenous gp100 peptide (Fig. 5*B*, 5*C*), indicating bortezomib had altered proteasomal processing and presentation of tumor Ags recognized by tumor-reactive CTLs. Although these data suggest bortezomib-treated tumors have reduced expression of Ags recognized by CTLs,

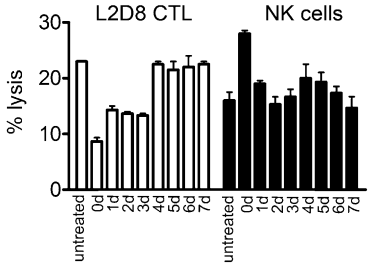

FIGURE 2. Short and long-term effects of tumor treatment with bortezomib on CTL and NK cell- mediated tumor lysis. 526mel tumors were treated with bortezomib (20 nM) for 18 h and then replaced with fresh media without bortezomib and cultured for another 1–7 d. Cells were thereafter tested for susceptibility to lysis by L2D8 cells (white bars) or NK cells (black bars) at an E:T ratio of 2.5:1.

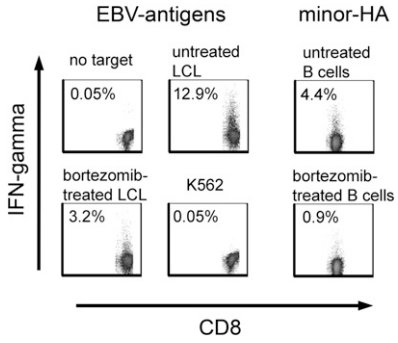

FIGURE 3. Reduced IFN-γ production in L2D8 CTLs in bortezomib-treated tumors. Intracellular staining for IFN-γ of EBV-specific T cells and minor histocompatibility (minor-HA) T cells in response to untreated and bortezomib-treated target cells. EBV-specific T cells were cocultured without targets, autologous EBV-transformed B cells, and K562 cells. Donor-derived minor histocompatibility Ag-specific T cells were cocultured with untreated or bortezomib-treated recipient B cells (obtained before undergoing an allogeneic MHC-matched stem cell transplantation; Protocol 99-H-0050). All cocultures were performed at a 2:1 E:T ratio for 5 h before staining for IFN-γ. *x*- and *y*-axis show log fluorescence. Gates were set for $CD8^+$ cells. Numbers in each graph represent the percentage of IFN-γ–positive cells.

imaging analysis using the ImageStream cytometer (Amnis) showed no reduction in the number of tumor/CTL conjugates when CTLs were cocultured with bortezomib-treated tumor cells compared with untreated tumors (data not shown). T cell-independent docking to target cells through receptors such as CD8 and LFA-1 have been shown to support peptide/MHC

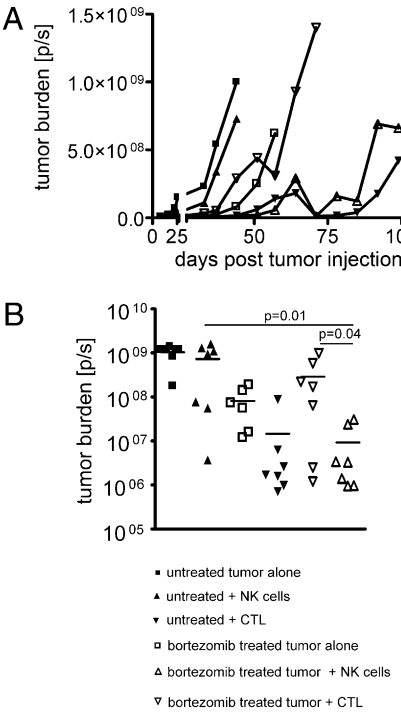

FIGURE 4. Rapid tumor progression in SCID beige mice upon injection of bortezomib-treated tumors and CTLs. *A*, 526mel cells containing luciferase were treated with bortezomib (20 nM, 18 h) and thereafter washed and plated at 0.5×10^6 cells/well in a six-well plate and cocultured with 1.5×10^6 human NK cells or L2D8 T cells for 6 h. Cell numbers of bortezomib-treated cells were adjusted to untreated cells before coculture. Mice ($n = 7$) were followed for tumor growth by bioluminescence imaging every 3–7 d starting 1 to 2 wk after tumor injection. Numbers indicate tumor doubling time. *B*, Tumor burden in mice on day 44 post tumor injection. Tumor burden is measured as total bioluminescence flux in photons per second (p/s).

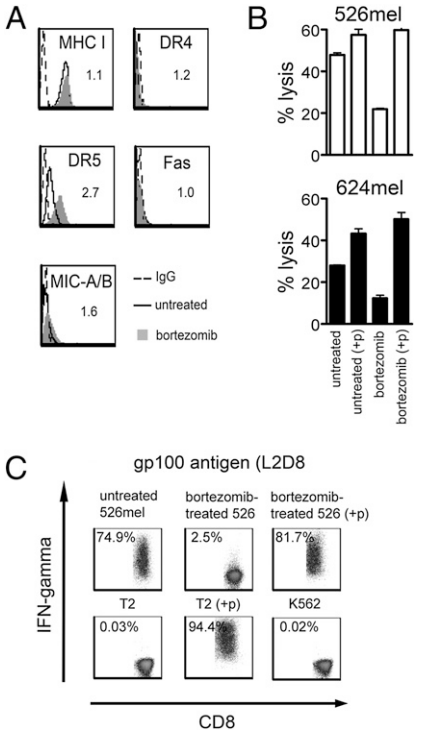

FIGURE 5. *A*, Restoration of T cell cytotoxicity and IFN-γ production by culturing bortezomib-treated tumors with exogenous gp100 peptide and 624mel cells were treated with bortezomib (20 nM) for 18 h and stained with Abs against MHC class I, DR4 (TRAIL-R1), DR5 (TRAIL-R2), Fas (CD95), and MIC-A/B. Similar results were obtained with the 526mel cell line (data not shown). Values show mean fluorescence intensity fold change in bortezomib-treated and untreated tumor cells. *x*-axis shows log fluorescence. *B*, Untreated and bortezomib-treated (20 nM, 18 h) 526mel and 624mel cells were preincubated with 1 mM of gp100 (209–217) peptide (+p) for 2 h at 37°C and tested for susceptibility to lysis by L2D8 T cells in a 4-h chromium release assay at an E:T ratio of 8:1. *C*, Intracellular staining for IFN-γ of gp100-specific T cells (L2D8) in response to untreated and bortezomib-treated target cells. L2D8 cells were cocultured with gp100-209 peptide-pulsed or unpulsed 526mel cells, T2 cells or K562 cells. All cocultures were performed at a 2:1 E:T ratio for 5 h before staining for IFN-γ. *x*- and *y*-axis show log fluorescence. Gates were set for $CD8^+$ cells. Numbers in each graph represent the percentage of IFN-γ–positive cells.

independent (i.e., noncognate) T cell adhesion, potentially accounting for the similarities in CTL binding to tumor targets (20, 21). In contrast to these results, Morishima et al. (18) reported recognition of cervical cancer cell lines by HPV-16 E6 (49–57) peptide-specific T cells was augmented when tumors were exposed to bortezomib. Their data suggest that a cryptic epitope might have been generated by bortezomib-induced nonproteasomal cytosolic proteases or by proteasome activity other than chymotryptic-like activity (18). In a B16 melanoma mouse model, Schumacher et al. (19) demonstrated

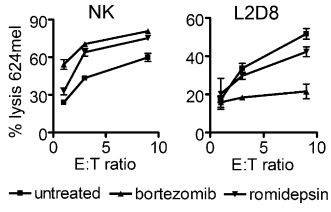

FIGURE 6. Romidepsin sensitizes tumors to NK cell-mediated lysis without decrease in T cell mediated lysis. Untreated, bortezomib-treated (20 nM, 18 h) or romidepsin-treated (25 ng/ml, 18 h) 624mel cells were tested for susceptibility to lysis by NK cells and L2D8 T cells in a 4-h chromium release assay.

bortezomib treatment combined with DC vaccination resulted in CD8 T cell- and NK cell-dependent tumor lysis. In their model, bortezomib treatment directly caused B16 melanoma cell apoptosis, potentially resulting in cross-presentation of tumor Ags to DCs, which primed $CD8^+$ tumor Ag-reactive T cells after bortezomib levels declined.

Preclinical studies have also shown bortezomib prevents graft-versus-host disease (GVHD) after allogeneic hematopoietic stem cell transplantation (20, 21). This reduction in T cell alloreactivity has been hypothesized to occur as a consequence of bortezomib inducing apoptosis in activated T cells and by inhibition of DC function. Our data showing T cell recognition of targets is reduced as a direct consequence of bortezomib altering proteasomal processing and presentation of Ags, including minor histocompatibility Ags, which provides an alternative mechanism whereby bortezomib might reduce GVHD when given after allogeneic transplantation. Remarkably, Sun et al. (20) showed that graft-versus-tumor (GVT) effects were maintained in bortezomib-treated animals that had reduced GVHD. However, these results were obtained in MHC-mismatched transplantation models, which have lesser clinical relevance to HLA-matched transplants in humans, where minor histocompatibility Ags rather than mismatched HLA molecules serve as the dominant targets for GVT effects. Our data showing bortezomib reduces minor histocompatibility specific T cell recognition as well as tumor Ag-specific T cell recognition raises the concern that GVT effects against tumors might be reduced when this agent is given following an HLA-matched hematopoietic cell transplantation. Alternatively, other agents, including the histone deacetylase inhibitor romidepsin, which sensitizes tumors to NK cell-mediated lysis without reducing Ag-specific T cell tumor cytotoxity (Fig. 6), could be used.

In conclusion, we found bortezomib-treated tumors sensitized to NK cell cytotoxicity simultaneously acquire resistance to Ag-specific T cell effector responses. Based on these observations, the use of bortezomib in conjunction with therapies aimed at bolstering Ag-specific T cell immunity against cancer should be approached with caution.

Acknowledgments

We thank Dr. M. Dudley (Surgery Branch, National Cancer Institute) for providing melanoma cell lines and reagents. We also thank Leigh Samsel and Phil McCoy at the National Heart, Lung, and Blood Institute flow cytometry core facility for assistance with ImageStream experiments. We also wish to acknowledge Action to Cure Kidney Cancer, The Dean R. O'Neill Memorial Fellowship, and Bill Rancic for generous contributions supporting this research.

Disclosures

The authors have no financial conflicts of interest.

References

1. Hernandez-Ilizaliturri, F. J., N. Reddy, B. Holkova, E. Ottman, and M. S. Czuczman. 2005. Immunomodulatory drug CC-5013 or CC-4047 and rituximab enhance antitumor activity in a severe combined immunodeficient mouse lymphoma model. *Clin. Cancer Res.* 11: 5984–5992.
2. Li, B., J. Lin, M. Vanroey, M. Jure-Kunkel, and K. Jooss. 2007. Established B16 tumors are rejected following treatment with GM-CSF-secreting tumor cell immunotherapy in combination with anti-4-1BB mAb. *Clin. Immunol.* 125: 76–87.
3. Butler, L. M., V. Liapis, S. Bouralexis, K. Welldon, S. Hay, M. le Thai, A. Labrinidis, W. D. Tilley, D. M. Findlay, and A. Evdokiou. 2006. The histone deacetylase inhibitor, suberoylanilide hydroxamic acid, overcomes resistance of human breast cancer cells to Apo2L/TRAIL. *Int. J. Cancer* 119: 944–954.
4. Lacour, S., A. Hammann, A. Wotawa, L. Corcos, E. Solary, and M. T. Dimanche-Boitrel. 2001. Anticancer agents sensitize tumor cells to tumor necrosis factor-related apoptosis-inducing ligand-mediated caspase-8 activation and apoptosis. *Cancer Res.* 61: 1645–1651.
5. Lundqvist, A., S. I. Abrams, D. S. Schrump, G. Alvarez, D. Suffredini, M. Berg, and R. Childs. 2006. Bortezomib and depsipeptide sensitize tumors to tumor necrosis factor-related apoptosis-inducing ligand: a novel method to potentiate natural killer cell tumor cytotoxicity. *Cancer Res.* 66: 7317–7325.
6. Watanabe, K., K. Okamoto, and S. Yonehara. 2005. Sensitization of osteosarcoma cells to death receptor-mediated apoptosis by HDAC inhibitors through down-regulation of cellular FLIP. *Cell Death Differ.* 12: 10–18.
7. Hallett, W. H., E. Ames, M. Motarjemi, I. Barao, A. Shanker, D. L. Tamang, T. J. Sayers, D. Hudig, and W. J. Murphy. 2008. Sensitization of tumor cells to NK cell-mediated killing by proteasome inhibition. *J. Immunol.* 180: 163–170.
8. Lundqvist, A., S. Rao, A. Smith, M. Berg, S. Su, H. Yokoyama, S. Srivastava, and R. Childs. 1786. 2007. Bortezomib enhanced the anti-tumor activity of adoptively infused natural killer cells in vivo a novel approach to override KIR-mediated inhibition of NK cell cytotoxicity. *Blood* 110: 530A–530A.
9. Sayers, T. J., A. D. Brooks, C. Y. Koh, W. Ma, N. Seki, A. Raziuddin, B. R. Blazar, X. Zhang, P. J. Elliott, and W. J. Murphy. 2003. The proteasome inhibitor PS-341 sensitizes neoplastic cells to TRAIL-mediated apoptosis by reducing levels of c-FLIP. *Blood* 102: 303–310.
10. Dudley, M. E., J. R. Wunderlich, T. E. Shelton, J. Even, and S. A. Rosenberg. 2003. Generation of tumor-infiltrating lymphocyte cultures for use in adoptive transfer therapy for melanoma patients. *J. Immunother.* 26: 332–342.
11. Takahashi, Y., J. P. McCoy, Jr., C. Carvallo, C. Rivera, T. Igarashi, R. Srinivasan, N. S. Young, and R. W. Childs. 2004. In vitro and in vivo evidence of PNH cell sensitivity to immune attack after nonmyeloablative allogeneic hematopoietic cell transplantation. *Blood* 103: 1383–1390.
12. Nencioni, A., K. Schwarzenberg, K. M. Brauer, S. M. Schmidt, A. Ballestrero, F. Grünebach, and P. Brossart. 2006. Proteasome inhibitor bortezomib modulates TLR4-induced dendritic cell activation. *Blood* 108: 551–558.
13. Straube, C., R. Wehner, M. Wendisch, M. Bornhäuser, M. Bachmann, E. P. Rieber, and M. Schmitz. 2007. Bortezomib significantly impairs the immunostimulatory capacity of human myeloid blood dendritic cells. *Leukemia* 21: 1464–1471.
14. Subklewe, M., K. Sebelin-Wulf, C. Beier, A. Lietz, S. Mathas, B. Dörken, and A. Pezzutto. 2007. Dendritic cell maturation stage determines susceptibility to the proteasome inhibitor bortezomib. *Hum. Immunol.* 68: 147–155.
15. Berges, C., H. Haberstock, D. Fuchs, M. Miltz, M. Sadeghi, G. Opelz, V. Daniel, and C. Naujokat. 2008. Proteasome inhibition suppresses essential immune functions of human CD4+ T cells. *Immunology* 124: 234–246.
16. Blanco, B., J. A. Pérez-Simón, L. I. Sánchez-Abarca, X. Carvajal-Vergara, J. Mateos, B. Vidriales, N. López-Holgado, P. Maiso, M. Alberca,E. Villarón et al. 2006. Bortezomib induces selective depletion of alloreactive T lymphocytes and decreases the production of Th1 cytokines. *Blood* 107: 3575–3583.
17. Orciuolo, E., S. Galimberti, and M. Petrini. 2007. Bortezomib inhibits T-cell function versus infective antigenic stimuli in a dose-dependent manner in vitro. *Leuk. Res.* 31: 1026–1027.
18. Morishima, S., Y. Akatsuka, A. Nawa, E. Kondo, T. Kiyono, H. Torikai, T. Nakanishi, Y. Ito, K. Tsujimura,K. Iwata et al. 2007. Identification of an HLA-A24-restricted cytotoxic T lymphocyte epitope from human papillomavirus type-16 E6: the combined effects of bortezomib and interferon-γ on the presentation of a cryptic epitope. *Int. J. Cancer* 120: 594–604.
19. Schumacher, L. Y., D. D. Vo, H. J. Garban, B. Comin-Anduix, S. K. Owens, V. B. Dissette, J. A. Glaspy, W. H. McBride, B. Bonavida, J. S. Economou, and A. Ribas. 2006. Immunosensitization of tumor cells to dendritic cell-activated immune responses with the proteasome inhibitor bortezomib (PS-341, Velcade). *J. Immunol.* 176: 4757–4765.
20. Sun, K., L. A. Welniak, A. Panoskaltsis-Mortari, M. J. O'Shaughnessy, H. Liu, I. Barao, W. Riordan, R. Sitcheran, C. Wysocki,J. S. Serody et al. 2004. Inhibition of acute graft-versus-host disease with retention of graft-versus-tumor effects by the proteasome inhibitor bortezomib. *Proc. Natl. Acad. Sci. USA* 101: 8120–8125.
21. Vodanovic-Jankovic, S., P. Hari, P. Jacobs, R. Komorowski, and W. R. Drobyski. 2006. NF-kappaB as a target for the prevention of graft-versus-host disease: comparative efficacy of bortezomib and PS-1145. *Blood* 107: 827–834.