

Combinatorial efficacy of anti-CS1 monoclonal antibody elotuzumab (HuLuc63) and bortezomib against multiple myeloma

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Abstract

Monoclonal antibody (mAb) therapy for multiple myeloma, a malignancy of plasma cells, has not been clinically efficacious in part due to a lack of appropriate targets. We recently reported that the cell surface glycoprotein CS1 (CD2 subset 1, CRACC, SLAMF7, CD319) was highly and universally expressed on myeloma cells while having restricted expression in normal tissues. Elotuzumab (formerly known as HuLuc63), a humanized mAb targeting CS1, is currently in a phase I clinical trial in relapsed/refractory myeloma. In this report we investigated whether the activity of elotuzumab could be enhanced by bortezomib, a reversible proteasome inhibitor with significant activity in myeloma. We first showed that elotuzumab could induce patient-derived myeloma cell killing within the bone mar-

row microenvironment using a SCID-hu mouse model. We next showed that CS1 gene and cell surface protein expression persisted on myeloma patient-derived plasma cells collected after bortezomib administration. *In vitro* bortezomib pretreatment of myeloma targets significantly enhanced elotuzumab-mediated antibody-dependent cell-mediated cytotoxicity, both for OPM2 myeloma cells using natural killer or peripheral blood mononuclear cells from healthy donors and for primary myeloma cells using autologous natural killer effector cells. In an OPM2 myeloma xenograft model, elotuzumab in combination with bortezomib exhibited significantly enhanced *in vivo* anti-tumor activity. These findings provide the rationale for a clinical trial combining elotuzumab and bortezomib, which will test the hypothesis that combining both drugs would result in enhanced immune lysis of myeloma by elotuzumab and direct targeting of myeloma by bortezomib. [Mol Cancer Ther 2009;8(9):2616–24]

Introduction

Considerable progress has been made in the management of multiple myeloma by the application of high-dose chemotherapy supported by autologous stem cell transplantation, which prolongs overall and disease-free survival (1). We recently reported that combining novel agents such as thalidomide and bortezomib with stem cell transplantation can substantially improve outcome and overcome the poor prognosis imparted by the *FGFR3/MMSET* translocation and 17p deletion (2). Monoclonal antibody (mAb) therapy is non-cross resistant with chemotherapy and has the potential for further improving results, as has been the case for rituximab in B-cell non-Hodgkin lymphomas. The CD-2 subgroup of myeloma frequently overexpresses CD20 (3), but therapy with rituximab has yielded disappointing results probably due to heterogeneous expression of CD20 and the presence of the complement regulator CD59 on myeloma, which renders complement-mediated cytotoxicity ineffective (4–7).

At present there are no mAbs approved for the treatment of multiple myeloma. A number of mAbs are under investigation in multiple myeloma targeting antigens such as CD40, (8–10) CD56, (11) CD74, (12, 13), and HM1.24 (14). We recently reported that the cell surface glycoprotein CS1 (CD2 subset 1, CRACC, SLAMF7, CD319), a member of the signaling lymphocyte activating-molecule-related receptor family, is selectively and uniformly expressed at high levels on myeloma cells independent of the presence of metaphase cytogenetic abnormalities or molecular subgroup (15). CS1 is not expressed by normal tissues or stem cells, with the exception of some lymphocyte subsets that have lower expression levels compared with malignant plasma cells (15).

Received 7/30/08; revised 6/4/09; accepted 6/18/09; published OnlineFirst 9/1/09.

Grant support: F. van Rhee is supported by NIH grants CA55819, CA134522, Multiple Myeloma Senior award 28-06 and funding from Facet Biotech Corp.

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Note: Supplementary material for this article is available at Molecular Cancer Therapeutics Online (<http://mct.aacrjournals.org/>).

F. van Rhee, D.E.H. Afar, S. Szmania, and S. Yaccoby designed the research; F. van Rhee, D.E.H. Afar, and S. Szmania wrote the paper; S. Szmania, S. Yaccoby, X. Li, J. Shi, M. Dillon, A. van Abbema, R. Yun, B. Balasa, B. Ganguly, A.G. Rice, F. Zhan, J.D. Shaughnessy, and D. Chao did research, collected data, and analyzed and interpreted data; F. van Rhee and B. Barlogie enrolled and treated patients on research protocols.

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doi:10.1158/1535-7163.MCT-09-0483

Interestingly, the *CS1* gene is located on chromosome 1q, amplifications of which are frequent in aggressive myeloma and linked to early myeloma-related death due to over-expression of the cell cycle regulator *CKS1B* (16).

We and others have shown that the humanized anti-*CS1* mAb elotuzumab exerts antimyeloma activity *in vitro* via antibody-dependent cellular cytotoxicity (ADCC) mediated by natural killer (NK) cells, and does not rely on complement-mediated cytotoxicity (15, 17). Elotuzumab has single-agent activity in myeloma xenograft models and is currently being tested in a phase I safety trial for relapsed/refractory myeloma (15, 17, 18). Recent work shows that down-regulation of the cell surface expression of MHC class I, an inhibitor of NK cell function, by bortezomib enhances the susceptibility of myeloma cells to NK cell-mediated killing (19). Furthermore, bortezomib-induced up-regulation of CD95 and TRAILR2 on tumor cells can lead to enhanced NK cell-mediated killing (20). We therefore hypothesized that the combination of elotuzumab and bortezomib may maximize NK cell-mediated destruction of multiple myeloma. In the present study, we first establish that elotuzumab is effective in the SCID-hu mouse model, which allows for the growth of primary myeloma cells in a human bone microenvironment. Further, we show that combining elotuzumab with bortezomib leads to strong antimyeloma activity both *in vitro* and *in vivo*.

Materials and Methods

Cells, Cell Lines, and Cultures

Peripheral blood was collected from healthy donors, and fresh blood or bone marrow was collected from patients with multiple myeloma after informed consent in accordance with the Declaration of Helsinki. Approval was obtained from the University of Arkansas for Medical Sciences Institutional Review Board for these studies. NK cells were enriched from whole blood samples either with the RosetteSep human NK cell enrichment cocktail (Stem-Cell Technologies Inc.) or using microbeads coated with CD56 mAb (Miltenyi Biotech). CD138-positive plasma cells were purified from patient-derived bone marrow aspirates via positive selection with anti-CD138 microbeads (Miltenyi Biotech). After magnetic bead selection, cells were confirmed to be >90% pure by flow cytometry for CD56 or CD138, respectively. The OPM2 and K562 cell lines were obtained from the American Type Culture Collection.

Reagents

Clinical grade bortezomib (Velcade) was purchased from or gifted by Millennium Pharmaceuticals, Inc. Anti-human *CS1* mAbs were generated as previously described, including murine IgG2a MuLuc63 and its humanized IgG1 version elotuzumab (formerly HuLuc63), both specific for the extracellular region of human *CS1*, and 1G9 mAb that recognizes the intracellular region (15). The human IgG₁ isotype control mAb, MSL109, used in all *in vitro* experiments, is a fully human anticytomegalovirus mAb (21). Murine IgG2a isotype control mAb, used for all *in vivo* experiments, is a mAb directed against VP7 of bluetongue virus and is available as a hybridoma from the American Type Culture Collection (clone 8A3B.6).

SCID-hu Mouse Model

C.B-17/IcrHsd-Prkdcscid (Harlan Sprague) were implanted s.c. with human fetal bone (Advanced Bioscience Resources) as previously described (22, 23). Upon bone engraftment, 2 to 8 × 10⁶ cells from heparinized bone marrow aspirates obtained from myeloma patients containing at least 20% CD138-positive plasma cells were injected directly into the human fetal bone in 50 μL PBS. For each experiment, cells from the same patient were injected into the fetal bone of five to six SCID-hu hosts. Changes in levels of circulating human immunoglobulin (hu Ig) of the M-protein isotype, monitored by ELISA as previously described (22), was used as an indicator of myeloma growth. When hu Ig levels reached 10 μg/mL or higher in two consecutive measurements, two mice with comparable tumor load (tumor derived from the same patient) were randomized to receive murine isotype control mAb (anti-bluetongue virus) or Mu-Luc63 (parental mouse anti-*CS1* mAb). Dosing was 10 mg/kg of mAb administered i.p. two times a week for a total of six doses. Tumor growth was monitored for a period of about 6 wk by ELISA for hu Ig. The statistical significance of differences was assessed with Student's *t* test, and *P* < 0.05 was considered significant. All animal work was carried out according to the NIH Guide for the Care and Use of Laboratory Animals, with protocols approved by the University of Arkansas for Medical Sciences Institutional Animal Care and Use Committee.

Gene Expression Profiling

CD138-positive plasma cells were purified from samples of bone marrow from patients with multiple myeloma, as described above, before and 48 h after a single dose of 1 mg/m² bortezomib. Gene expression profiling for *CS1* expression (probe set 219159_s_at) was done using the Affymetrix U133 Plus 2.0 arrays platform (Affymetrix Inc.) as previously described (24). Student's *t* test was used to determine whether *CS1* gene expression was significantly different in plasma cells collected before versus after bortezomib administration.

Flow Cytometry

CD138-purified multiple myeloma cells from bone marrow collected from patients before and 48 h after a dose of 1 mg/m² bortezomib were purified as above, cryopreserved, then subsequently thawed and analyzed in the same experiment by flow cytometry for *CS1* expression. Dead and apoptotic cells were gated out using propidium iodide and Annexin V stains (Invitrogen), and the resulting viable cell population was analyzed for *CS1* expression using elotuzumab conjugated to FITC. Samples were run on a FACSARIA cytometer (BD Pharmingen). Histogram overlays were created using FCS express software (De Novo Software).

ADCC Assay

Target cells were suspended at a density of 1 × 10⁶ cells/mL in RPMI media with a fixed dose of 10 nmol/L bortezomib or titrated doses of bortezomib (20 nmol/L, 10 nmol/L, 5 nmol/L, 1 nmol/L) or in media alone and incubated in a 5% CO₂ incubator at 37° for 18 h. Cells were collected, washed, assessed for viability, resuspended at a density of

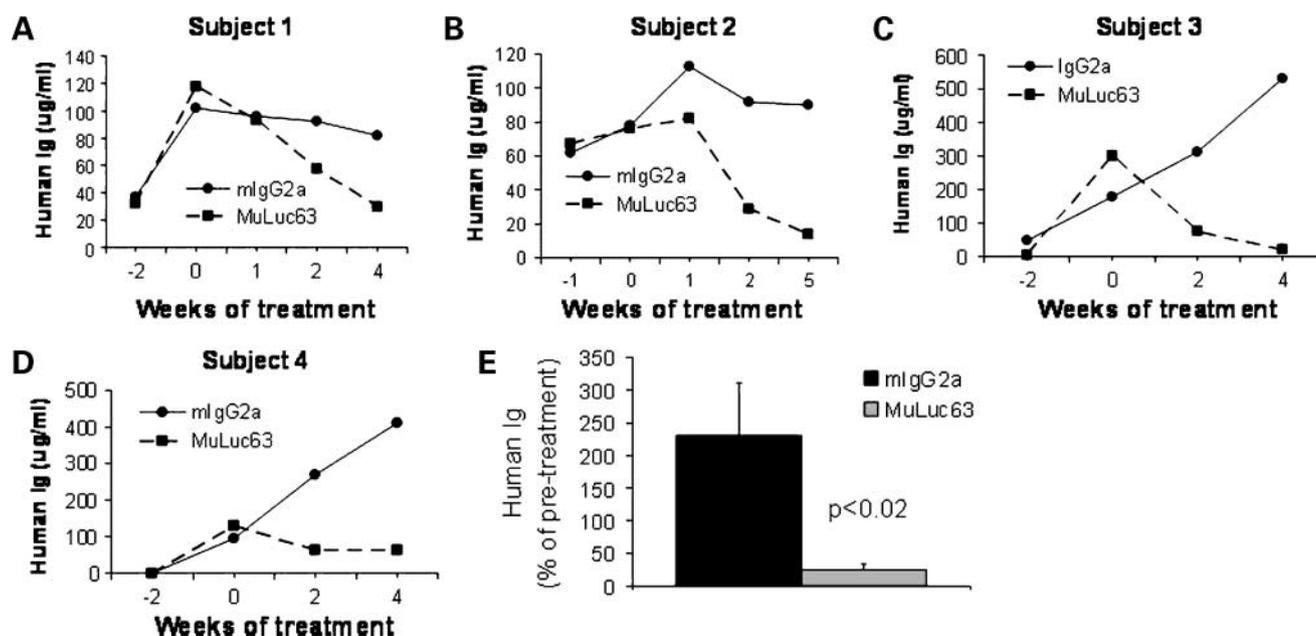


Figure 1. Anti-CS1 mAb MuLuc63 induces marked reduction of primary human myeloma tumors in the SCID-hu host. **A** to **D**, each individual experiment uses patient-derived myeloma injected into an implanted human bone allowed to grow, then treated with anti-CS1 mAb MuLuc63 or murine isotype control mIgG2a antibody. A dramatic reduction in tumor load was observed in each experiment. **E**, the difference of hu Ig detected (calculated as the percent of pretreatment) for MuLuc63-treated versus control mIgG2a mAb is significantly different (Student's *t* test).

20×10^6 viable cells/mL, and labeled for 1 h with 50 mCi Na_2 ^{51}Cr O_4 per 10^6 cells. ^{51}Cr -labeled cells were washed and added to a 96-well, V-bottomed polystyrene plate at a cell density of 15,000 cells per 75 μL . Elotuzumab or MSL109 (isotype control mAb) was added in a volume of 25 μL to the target cells for a final concentration of 0.001 to 10 $\mu\text{g}/\text{mL}$. NK cells or peripheral blood mononuclear cells (PBMC) from healthy donors were added in a volume of 100 μL to bortezomib-treated or mock-treated target cells at a ratio of 10:1 (NK:targets) or 25:1 (PBMC:targets). Pretreatment of targets for 0.5 h with 50 mcg/mL Fc receptor blocking antibody (Serotec) was done for some assays. After a 4-h incubation at 37°C, cells were centrifuged at 1,200 rpm for 5 min, and released ^{51}Cr was measured in the 100 μL supernatants. Maximum release was determined from target cells lysed with 100 mg/mL digitonin or 1% Triton X-100. Antibody-independent cellular cytotoxicity (AICC) was determined using target cells, plus media, plus NK cells; spontaneous lysis was determined using ^{51}Cr -labeled cells plus media without NK or PBMC effectors. Percent cytotoxicity was calculated as $([\text{sample} - \text{AICC}] / [\text{Maximum} - \text{AICC}]) \times 100$. The concentration of elotuzumab necessary to produce 50% of the maximal elotuzumab-mediated ADCC response (EC_{50}) was determined from a curve fit using the sigmoidal dose-response non-linear curve regression in GraphPad Prism. EC_{50} values from the untreated cells were compared with those cells treated with bortezomib in the same experiment. A two-tailed paired *t*-test was used to determine the *P* value.

Primary myeloma cells were purified from patient bone marrow, as described above, and treated with 5 or

20 nmol/L bortezomib or vehicle for 18 h to assess the effects of bortezomib on elotuzumab-mediated lysis of patient-derived autologous NK cells. Cr^{51} release assays were done as above with NK cell:myeloma cell ratios of 1:1, 10:1, and 30:1. Elotuzumab and isotype control mAb MSL109 were used at a concentration of 10 $\mu\text{g}/\text{mL}$. The NK cell-sensitive cell line K562 was included as a positive control. Percent cytotoxicity was calculated as $([\text{sample} - \text{spontaneous lysis}] / [\text{Maximum} - \text{spontaneous lysis}]) \times 100$. *In vitro* experiments were done in triplicate, and the results are reported as mean \pm SE.

Mouse Xenograft Model

Female IcrTac:ICR-Prkdc^{scid} mice (6–8 weeks of age) were obtained from Taconic Farms and were inoculated in the lower right flank with 1×10^7 OPM2 cells (American Type Culture Collection) in RPMI-1640 media from HyClone. Caliper measurements were done thrice weekly for the calculation of tumor volume, and tumor growth was monitored for a period of 1 to 2 mo. The following formula was used to calculate tumor volume: $L \times W \times H/2$, where *L* (length) is the longest side of the tumor in the plane of the animal's back, *W* (width) is the longest measurement perpendicular to the length and in the same plane, and *H* (height) is the highest point perpendicular to the back of the animal.

Mice with an average tumor size of 100 mm^3 were allocated randomly into treatment groups of 10 to 15 mice each. Bortezomib was administered i.p. in PBS at a dose of 1 mg/kg or twice weekly for weeks 1 and 2, no treatment for week 3, and 1 mg/kg twice weekly for weeks 4 and 5 (total of 8 doses). Elotuzumab or MSL 109 (at doses of 1 or 10 mg/kg

in PBS) were administered i.p. twice weekly for 5 wk (total of 10 doses) on days offset from bortezomib dosing. One-way ANOVA with a Tukey posttest was used to determine differences between elotuzumab and control treatment groups. All animal work was carried out according to the NIH Guide for the Care and Use of Laboratory Animals, with protocols approved by the Institutional Animal Care and Use Committee at PDL BioPharma.

Immunohistochemistry

Engrafted tumor with human fetal bones in the SCID-hu model were removed, formalin fixed for 24 h and then decalcified with EDTA, pH 7.0. Decalcified, formalin-fixed, paraffin-embedded tissues were cut into 4- μ m/L sections for H&E staining. OPM2 xenograft tumors were harvested at the end of study and fixed in formalin for 24 h. Deparaffined tissue slides were immersed in DAKO Target retrieval solution (pH 6) in a decloaking chamber (Biocare Medical) for antigen epitope retrieval. CS1 expression in OPM2 xenografts and primary myeloma tumor grafts was determined by staining the tissues with 1G9 mouse mAb at 1 μ g/mL for 30 min and EnVision secondary polymer System (Dako). Magnification is at 50 \times . Percent tumor burden was determined by a certified pathologist using histologic analysis of the tumor grafts.

Results

Elotuzumab Induces Killing of Primary Myeloma Cells in the SCID-hu Mouse Model

Elotuzumab exhibits significant *in vivo* antitumor activity towards myeloma cell lines, inoculated s.c. in SCID mice (15, 17). To determine whether anti-CS1 therapy can inhibit the growth of primary myeloma cells in the human bone microenvironment, primary myeloma cells were injected into s.c. implanted human fetal bones engrafted in SCID mice. For each experiment five to six SCID-hu mice were injected with bone marrow from the same patient allowing for the selection of two mice with comparable tumor volumes, which were randomly allocated to MuLuc63 (the parental mAb from which elotuzumab was derived) or control mAb treatment. The mouse parental MuLuc63 mAb was used in these experiments to facilitate the measurement of circulating human IgG in the mice. We observed a statistically significant reduction of tumor volume and circulating human IgG levels in mice treated with MuLuc63, compared with the isotype control mAb (Fig. 1). Two of the four patients studied (subjects 2 and 3) had high-risk myeloma based on the 70-gene model, and responded equally well to MuLuc63 monotherapy as low-risk disease (subjects 1 and 4; ref. 16). Histologic analysis of the fetal bones (in 3 of 3 evaluable cases)

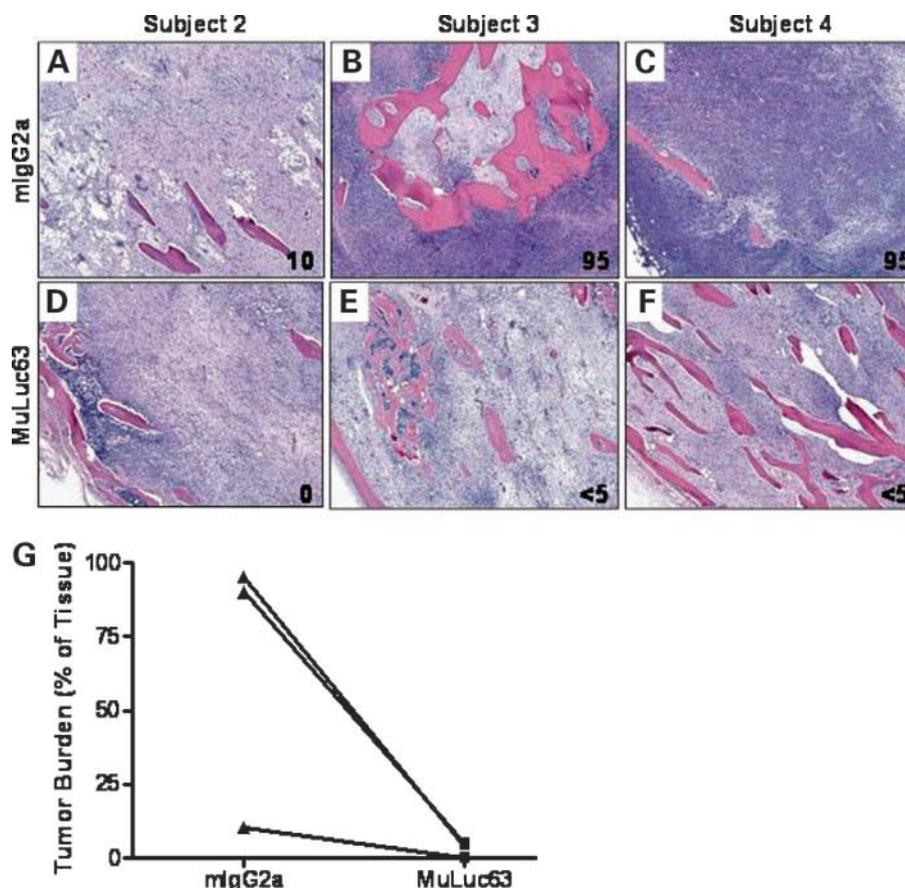


Figure 2. Histologic analysis of tumor burden with anti-CS1 therapy. The tumor burden in isotype control (mIgG2a)-treated mice versus MuLuc63-treated mice was calculated as the percent of tissue comprising myeloma cells and is shown for the three evaluable subjects. Histologic analysis showed a dramatic reduction in tumor burden in MuLuc63-treated (D–F) versus isotype control-treated mice (A–C). G, the tumor burden was scored by a certified pathologist.

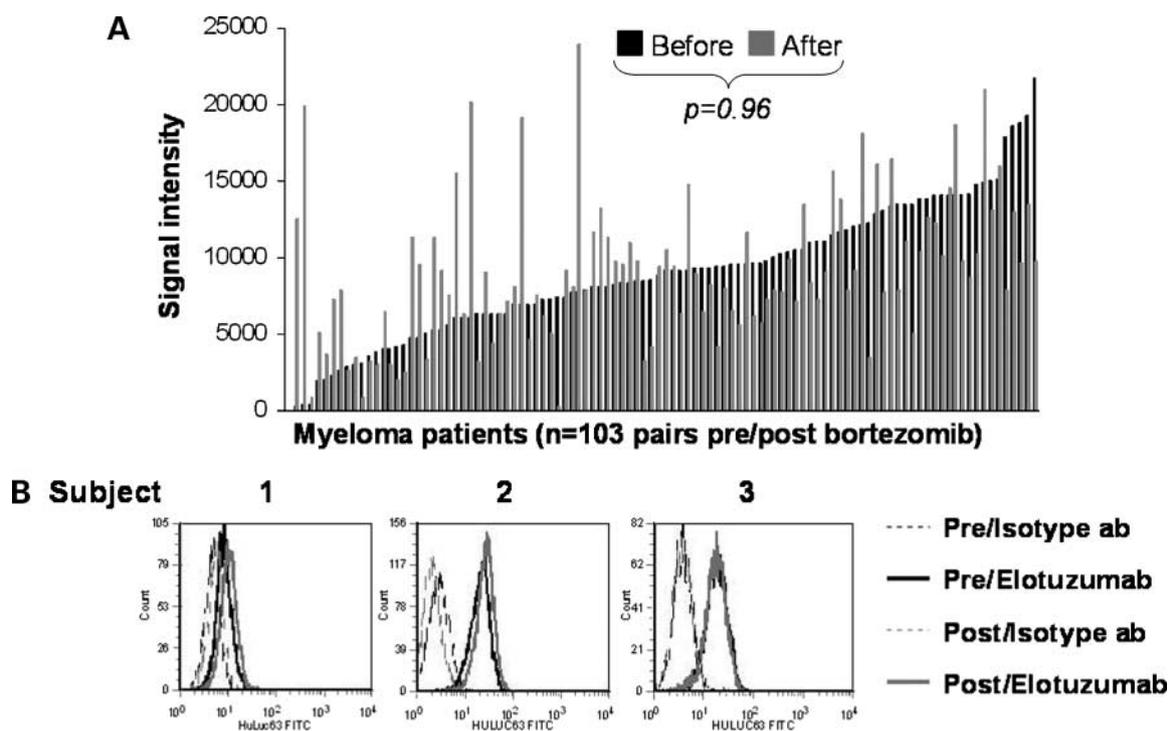


Figure 3. CS1 gene expression persists in patients with multiple myeloma after bortezomib treatment. **A**, CS1 gene expression profiling of CD138-positive myeloma cells purified from bone marrow before and 48 h after administration of 1 mg/m² bortezomib. Paired signal intensities are shown for 103 newly diagnosed myeloma patients. Student's *t* test was done to determine whether differences were significantly different. **B**, flow cytometry analysis of purified CD138-positive cells showed that cell surface CS1 expression remains present 48 h postbortezomib. A gate was applied which only captured Annexin V- and propidium iodide-negative CD138 purified myeloma cells.

showed nearly complete eradication of multiple myeloma in the MuLuc63 group, whereas multiple myeloma effaced the marrow in the control mAb group (Fig. 2). These results with MuLuc63 suggest that elotuzumab would exhibit significant antimyeloma activity in the bone marrow microenvironment.

CS1 RNA and Protein Expression Persists after Bortezomib Administration

Because one of the goals of the present study was to determine the effect of combining elotuzumab with bortezomib on antitumor activity, the effect of bortezomib on CS1 expression was examined. The gene expression profile of primary myeloma cells from 103 previously untreated patients both before and after a single dose of 1 mg/m² bortezomib was examined. No significant difference in mean CS1 gene expression level ($P = 0.96$; Fig. 3A) was observed. Furthermore, flow cytometry analysis showed that bortezomib did not alter the level of CS1 protein expression at the myeloma cell surface (Fig. 3B).

Bortezomib Enhances Elotuzumab-Mediated ADCC of CS1-Positive Myeloma Cell Lines and Primary Myeloma Cells

There was a dose-dependent increase in elotuzumab-mediated ADCC of the myeloma cell line OPM2, which was enhanced by pretreatment with 10 nmol/L bortezomib (Fig. 4A). The EC₅₀ of elotuzumab-mediated ADCC was decreased significantly by bortezomib in four in-

dependent experiments with NK cells from normal donors. Bortezomib also significantly ($P < 0.0006$) increased elotuzumab-induced lysis of the CS1-positive cell line XG-1, whereas the CS1-negative and bortezomib-resistant cell line RPMI8226/R5 was insensitive to treatment with either drug (Fig. 4B). Preincubation of effectors with FcR blocking antibody abrogated elotuzumab-induced killing, supporting the ADCC mechanism. When OPM2 cells were pretreated with varying doses of bortezomib (1, 5, 10, 20 nmol/L), significant enhancement of elotuzumab-mediated ADCC was observed only at doses of ≥ 5 nmol/L (results shown for 5 nmol/L dose in Fig. 4C). A dose of 1 nmol/L bortezomib had no significant effect on ADCC enhancement. Also, no significant difference was observed for 5, 10, and 20 nmol/L, suggesting that a threshold level of 5 nmol/L bortezomib is enough for maximal enhancement for OPM2 cells (data not shown). Patient-derived NK cells induced approximately 20% specific lysis of autologous myeloma cells in the presence of elotuzumab (10 μ g/mL) alone at a ratio of 30 NK cells to 1 multiple myeloma cell (Fig. 4D). A dose-dependent increase in specific lysis was observed when adding bortezomib. The CS1-mediated lysis of primary myeloma cells was similar to killing of the NK cell-sensitive cell line K562 at a dose of 20 nmol/L of bortezomib. This increase was not noted when isotype control mAb was added, suggesting that bortezomib sensitized primary myeloma cells specifically to elotuzumab-mediated ADCC.

Elotuzumab in Combination with Bortezomib Exhibits Significantly Enhanced Antimyeloma Activity Towards the OPM2 Xenograft Model

Elotuzumab has previously been shown to exert significant dose-dependent antimyeloma activity in mouse xenograft models (15, 17). We next investigated whether elotuzumab combined with bortezomib exhibit enhanced *in vivo* antitumor activity using the OPM2 xenograft tumor model (Fig. 5). The mice were randomized into 4 groups of 15 animals when the OPM2 tumors reached an average of

graft models (15, 17). We next investigated whether elotuzumab combined with bortezomib exhibit enhanced *in vivo* antitumor activity using the OPM2 xenograft tumor model (Fig. 5). The mice were randomized into 4 groups of 15 animals when the OPM2 tumors reached an average of

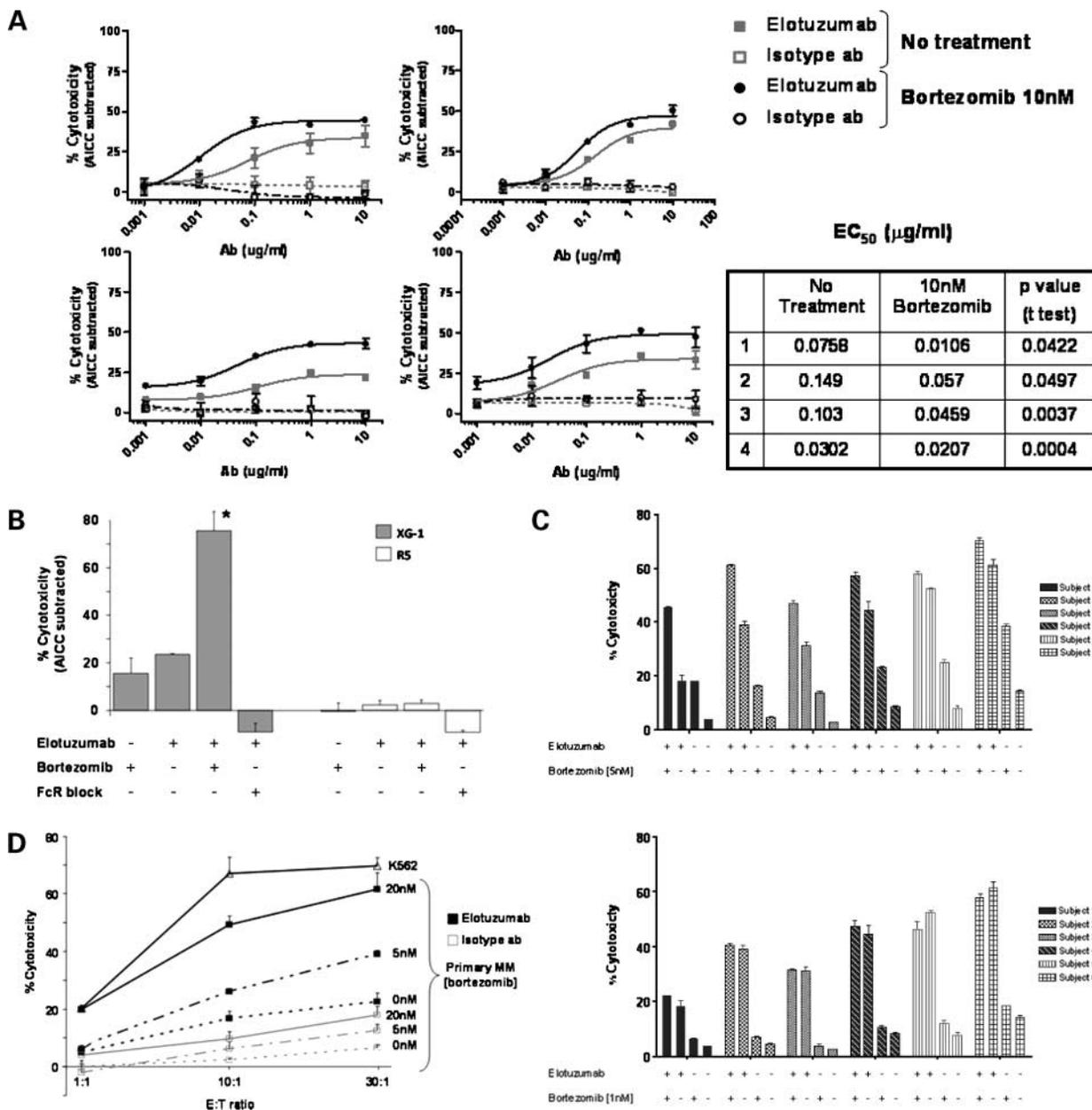


Figure 4. Bortezomib pretreatment enhances elotuzumab-mediated ADCC. **A**, results are shown for ADCC assays conducted with NK cells from four separate healthy donors against the OPM2 myeloma cell line. Results are expressed as mean \pm SD. **B**, results of ADCC assays using PBMC effectors from a healthy donor versus the myeloma cell lines XG-1 and RPMI8226/R5 at a ratio of 40 effectors:1 target cell. Results are expressed as the mean with SD. *, Student's *t* test determined that lysis of XG1 targets treated with both elotuzumab and bortezomib was significantly higher ($P < 0.006$) than elotuzumab or bortezomib alone. **C**, results for reactions conducted with PBMCs from six healthy individuals against OPM2 cells that were incubated for 18 h with vehicle, 5 nmol/L (*top*) or 1 nmol/L (*bottom*) bortezomib as indicated. Results (% cytotoxicity) are expressed as mean \pm SD. A dose of 5 nmol/L bortezomib consistently enhanced elotuzumab-mediated ADCC compared with vehicle treatment ($P = 0.0057$). A dose of 1 nmol/L bortezomib was not significantly different from vehicle ($P = 0.731$). **D**, results for reactions conducted with patient-derived NK cells against autologous myeloma cells that were incubated for 18 h with vehicle (*dotted lines*), 5 nmol/L bortezomib (*dashed/dotted lines*), or 20 nmol/L bortezomib (*solid lines*). Targets were also incubated with either isotype control mAb (\square) or elotuzumab (\blacksquare) during the assay. K562 cells (Δ) were included as a positive control. Results are expressed as mean \pm SE.

100 mm³. The mice were injected i.p. twice weekly with a previously established suboptimal dose of elotuzumab (1 mg/kg) or control mAb. This dose was 10-fold lower than the dose of elotuzumab that achieves optimal antitumor activity using the OPM2 xenograft model (17). Bortezomib or PBS was administered twice weekly for weeks 1 and 2, no treatment on week 3, and twice weekly during weeks 4 and 5. The dose of bortezomib was 1 mg/kg, which when given twice a week nears the maximum tolerated dose in these mice (data not shown). There was rapid tumor growth in mice treated with control mAb. Modest but significant anti-OPM2 activity was observed in the two groups treated with bortezomib alone ($P < 0.001$) or elotuzumab alone ($P < 0.001$). In contrast, there was a profound retardation of OPM2 tumor growth when mice were treated with elotuzumab combined with bortezomib. At day 38 of the study (the day at which the control group was terminated), mice in the combination treatment group exhibited mean tumor volumes 85% smaller than in the elotuzumab monotherapy group ($P < 0.001$), and 82% smaller than in the bortezomib monotherapy group ($P < 0.001$). At day 45 (the day on which the monotherapy groups were terminated), the combination therapy inhibited mean tumor volumes by 89% ($P < 0.001$) and 87% ($P < 0.001$) compared with elotuzumab and bortezomib monotherapy, respectively. This experiment was done three times, with similar results observed in all cases. When elotuzumab was used at the optimal dose of 10 mg/kg given twice weekly, the combination with bortezomib was not significantly more efficacious than the combination with 1 mg/kg of antibody (see supplementary data). This suggests that maximal combinatorial effect is already achieved when elotuzumab is used at the suboptimal dose of 1 mg/kg. Immunohistochemistry analysis of OPM2 tumors from mice with the noncompeting anti-CS1 mAb 1G9 showed that the expression of CS1 protein *in vivo* was not affected by bortezomib treatment (data not shown), in agreement with the results shown for myeloma cells from patients treated with bortezomib. These findings substantiate the notion that elotuzumab in combination with bortezomib exhibits significantly enhanced antitumor activity.

Discussion

CS1, a cell surface glycoprotein of the CD2 family, is highly and uniformly expressed by myeloma cells and therefore

merits investigation as a potential new target for myeloma therapy. Recent studies suggest that CS1 localizes to the uropods of polarized myeloma cells, suggesting a potential role for CS1 in mediating adhesion of myeloma cells to bone marrow stroma (25). CS1 also seems to protect myeloma cell lines from apoptosis by decreasing phosphorylation of ERK1/2, AKT, and STAT as well as modulating other proapoptotic and antiapoptotic pathways (17). Preliminary data suggest that CS1-specific cytotoxic T cells can kill both CS1 peptide pulsed T2 cells and the myeloma cell line MCCAR (26). Several lines of evidence suggest that the CS1-specific humanized mAb elotuzumab kills multiple myeloma via ADCC mediated by NK cells. Blocking of the Fc receptor on NK cells abrogated the antimyeloma effect of elotuzumab. Further, *in vivo* NK cell depletion in mouse xenograft models substantially reduces elotuzumab activity. Lastly, elotuzumab has no significant antitumor activity in NOD-SCID/IL2R γ knockout mice, which are deficient in NK cells (15). Because bortezomib has been shown to increase NK cell-mediated lysis of primary myeloma cells by down-regulating HLA-C, the principal ligand for inhibitory KIR receptors on NK cells (19), it was hypothesized that bortezomib might enhance the antimyeloma activity of elotuzumab.

Elotuzumab delays or abrogates the growth of human myeloma cell lines in several mouse xenograft models. However, most primary myeloma cells are dependent on stimuli provided by the bone marrow microenvironment and do not grow outside the skeleton in patients. As a result it has proven very difficult to grow primary myeloma cells in SCID-hosts, which limits extrapolation of results obtained with cell lines to the human setting. We therefore first established that elotuzumab has *in vivo* activity against primary myeloma cells by injecting bone marrow from myeloma patients into human fetal bones engrafted into SCID-hosts. In this model, growth of the primary myeloma cells was confined to human bone and mice developed classical myeloma symptoms such as bone marrow plasmacytosis, circulating monoclonal immunoglobulins, and severe resorption of fetal bone (22). Administration of the parental mouse antibody of elotuzumab (MuLuc63) for only 3 weeks reduced paraprotein levels and significantly reduced tumor burden in all animals at a dose level that is clinically achievable in humans

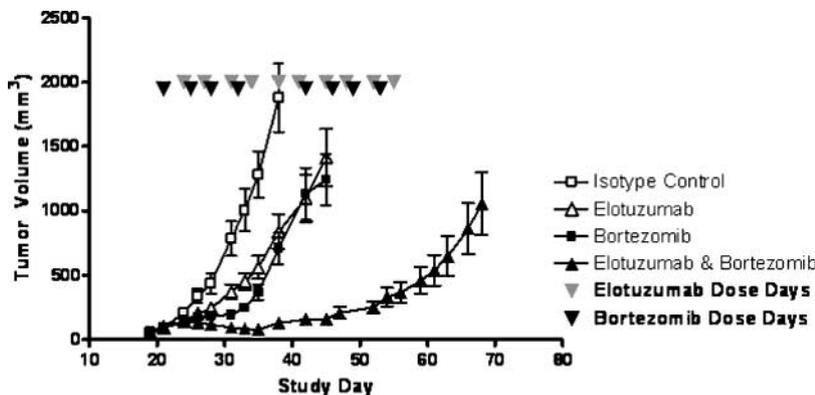


Figure 5. Bortezomib enhances elotuzumab-mediated antitumor activity in the OPM2 mouse xenograft model. OPM2 tumor-bearing mice (4 groups of $n = 15$ each) were treated with elotuzumab plus bortezomib, bortezomib plus isotype control mAb, PBS plus elotuzumab, and PBS plus isotype control mAb. Suboptimal doses of elotuzumab (1 mg/kg) or isotype control were administered i.p. twice weekly and bortezomib 1 mg/kg was administered i.p. twice weekly. Tumor volumes are expressed as mean \pm SD.

and comparable with concentrations found in the serum of patients treated with therapeutic doses of rituximab (27). Histologic analysis confirmed that virtually all myeloma cells were abolished in the MuLuc63-treated animals, whereas the marrow was replaced by myeloma cells in the control mAb group.

We next examined whether the combination of elotuzumab and bortezomib has enhanced antimyeloma activity. Administration of a single "test" dose of bortezomib at 1 mg/m² has been shown to significantly alter the gene expression profile of both primary myeloma cells and the human bone marrow environment (28). Our results showed that there was no significant alteration in CS1 RNA expression and CS1 protein expression at the cell surface after administration of clinically relevant bortezomib doses to a large cohort of untreated myeloma patients. Combination therapy of elotuzumab with bortezomib *in vitro* significantly lowered the EC₅₀ of elotuzumab in experiments with the cell line OPM2 and allogeneic effector cells from normal donors. Experiments targeting the CS1-positive, bortezomib-sensitive myeloma cell line XG-1 showed that bortezomib significantly increased killing of elotuzumab treated targets, whereas neither drug induced lysis of the bortezomib-resistant, CS1-negative myeloma line RPMI8226/R5. Further, increasing doses of bortezomib produced modest increases in killing of primary myeloma cells by autologous NK cells in the presence of control mAb. In contrast, the combination of bortezomib and elotuzumab augmented killing of primary myeloma cells to a level similar to lysis of the NK cell-sensitive positive control K562. In the OPM2 xenograft model combination therapy with elotuzumab and bortezomib induced significantly better tumor growth inhibition in comparison with application of either agent alone. Taken together these data support the notion that cotreatment of elotuzumab with bortezomib produces maximal antimyeloma activity. These findings are in keeping with recent observations by Tai et al. in which bortezomib enhanced elotuzumab-induced ADCC of the myeloma cell line MM1R by NK cells from healthy donors (17).

In summary, this study showed significant anti-CS1 mAb efficacy against patient-derived myeloma tumors in a human bone microenvironment. Furthermore, bortezomib significantly potentiated the effects of elotuzumab, possibly by rendering myeloma more vulnerable to NK cell-mediated lysis. These studies form the basis for clinical studies of combined elotuzumab and bortezomib therapy utilizing a novel approach in which myeloma cells are targeted directly by bortezomib, while at the same time enhancing the immune activity of elotuzumab. With the increased availability of drugs that target independent pathways involved in the pathophysiology of multiple myeloma, the goal of future investigations will be to determine those agents with the best synergism to be used for combination therapy to provide the greatest clinical benefits for patients suffering from this disease (29, 30).

Disclosure of Potential Conflicts of Interest

D.E.H. Afar, M. Dillon, A. van Abbema, R. Yun, B. Balasa, B. Ganguly, A.G. Rice, and D. Chao are current or former employees at Facet Biotech Corporation (formerly PDL BioPharma), F. van Rhee has received research funding from PDL BioPharma, B. Barlogie has received research funding from Millenium Pharmaceuticals.

Acknowledgments

We thank Tina Emek for excellent technical assistance; Gary Starling, Matt Williams and Debbie Law for critical review of the manuscript; and Lisa Shannon, PharmD of Scientific Connexions, for editing services.

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2624 Anti-Myeloma Activity of Elotuzumab Plus Bortezomib

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Mol Cancer Ther 2009;8:2616-2624. Published OnlineFirst September 1, 2009.

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