The Monoclonal Antibody nBT062 Conjugated to Cytotoxic Maytansinoids Has Selective Cytotoxicity Against CD138-Positive Multiple Myeloma Cells In vitro and In vivo

Hiroshi Ikeda,1,2 Teru Hideshima,1 Mariateresa Fulciniti,1 Robert J. Lutz,3 Hiroshi Yasui,1,2 Yutaka Okawa,1 Tanyel Kiziltepe,1 Sonia Vallet,1 Samantha Pozzi,1 Loredana Santo,1 Giulia Perrone,1 Yu-Tzu Tai,1 Diana Cirstea,1 Noopur S. Raje,1 Christoph Uherek,4 Benjamin Dälken,4 Silke Aigner,4 Frank Osterroth,4 Nikhil Munshi,1 Paul Richardson,1 and Kenneth C. Anderson1

Abstract Purpose: We investigated the antitumor effect of murine/human chimeric CD138-specific monoclonal antibody nBT062 conjugated with highly cytotoxic maytansinoid derivatives against multiple myeloma (MM) cells in vitro and in vivo.

Experimental Design: We examined the growth inhibitory effect of BT062-SPDB-DM4, BT062-SMCC-DM1, and BT062-SPP-DM1 against MM cell lines and primary tumor cells from MM patients. We also examined in vivo activity of these agents in murine MM cell xenograft model of human and severe combined immunodeficient (SCID) mice bearing implant bone chips injected with human MM cells (SCID-hu model).

Results: Anti-CD138 immunoconjugates significantly inhibited growth of MM cell lines and primary tumor cells from MM patients without cytotoxicity against peripheral blood mononuclear cells from healthy volunteers. In MM cells, they induced G2-M cell cycle arrest, followed by apoptosis associated with cleavage of caspase-3, caspase-8, caspase-9, and poly(ADP-ribose) polymerase. Nonconjugated nBT062 completely blocked cytotoxicity induced by nBT062-maytansinoid conjugate, confirming that specific binding is required for inducing cytotoxicity. Moreover, nBT062-maytansinoid conjugates blocked adhesion of MM cells to bone marrowstromal cells. The coculture of MM cells with bone marrow stromal cells protects against dexamethasone-induced death but had no effect on the cytotoxicity of immunoconjugates. Importantly, nBT062-SPDB-DM4 and nBT062-SPP-DM1 significantly inhibited MM tumor growth in vivo and prolonged host survival in both the xenograft mouse models of human MM and SCID-hu mouse model.

Conclusion: These results provide the preclinical framework supporting evaluation of nBT062-maytansinoid derivatives in clinical trials to improve patient outcome in MM.

The cell surface proteoglycan CD138 (syndecan-1) is an integral membrane protein acting as a receptor for the extracellular matrix. Within the normal human hematopoetic compartment, CD138 is expressed on differentiated plasma cells and is a primary diagnostic marker of multiple myeloma (MM; ref. 1). The large extracellular domain of CD138 binds via its heparin sulfate chains to soluble extracellular molecules, including the growth factors epidermal growth factor, fibroblast growth factor, and hepatocyte growth factor, and to insoluble extracellular molecules, such as collagen and fibronectin (2, 3). CD138 also mediates cell-cell adhesion through interactions with heparin-binding molecules. Studies of plasma cell differentiation show that CD138 is a differentiation antigen (4) and a coreceptor for MM growth factors (5). Several monoclonal antibodies (mAb; i.e., B-B4, BC/B-B4, B-B2, DL-101, 1 D4, M115, 1.BB.210, 2Q1484, 5F7, 104-9, 281-2) specific for CD138 have been reported. B-B4, 1D4, and M115 antibodies, which bind to similar or closely related epitopes,
Translational Relevance

CD138 is expressed on differentiated plasma cells and is a primary diagnostic marker of multiple myeloma (MM). In this study, we investigated the antitumor effect of murine/human chimeric CD138-specific monoclonal antibody nBT062 conjugated with highly cytotoxic maytansinoid derivatives against MM cells in vitro and in vivo. We first examined the growth inhibitory effect of BT062-SPDB-DM4, BT062-SMCC-DM1, and BT062-SPP-DM1 against MM cell lines and primary tumor cells from MM patients. We then examined in vivo activity of these agents in murine of human MM xenograft model and SCID-hu mice model, in which human MM cells injected into fetal bone chips implanted s.c. in severe combined immunodeficient mice. Importantly, nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPP-DM1 all have antitumor activity against MM cells and can overcome the protective effects of cytokines and bone marrow stromal cells. Our results, therefore, provide the preclinical framework for clinical trials of these agents to improve patient outcome in MM.

Materials and Methods

Cell culture. Dexamethasone-sensitive (MM.1S) and resistant (MM.1R) human MM cell lines were kindly provided by Dr. Steven Rosen (Northwestern University). RPMI8226, MOLP-8, and U266 human MM cell lines were obtained from American Type Culture Collection. Doxorubicin-resistant (RPMI-DOX40) and Melphalan-resistant (LR5) MM cell lines were kindly provided by Dr. William Dalton (Lee Moffitt Cancer Center). OPM1, INA-6, and OPM2 plasma cell leukemia cell lines were kindly provided by Dr. Edward Thompson (University of Texas Medical Branch). All MM cell lines were cultured in RPMI 1640 (Sigma), and bone marrow (BM) stromal cells were cultured in DMEM (Sigma) containing 10% fetal bovine serum, 2 mmol/L L-glutamine (Life Technologies), 100 units/mL penicillin, and 100 mg/mL streptomycin (Life Technologies). The interleukin-6 (IL-6)–dependent INA-6 cell line was cultured in the presence of 1.0 ng/mL of human recombinant IL-6 (R&D Systems). Blood samples collected from healthy volunteers were processed by Ficoll Paque centrifugation to obtain peripheral blood mononuclear cells (PBMC). Cell lines were harvested from BM samples after informed consent was obtained per the Declaration of Helsinki and approval by the Institutional Review Board of the Dana-Farber Cancer Institute. Normal donor BM mononuclear cells were separated by Ficoll Paque density sedimentation, and plasma cells were purified (>95% CD138+) by positive selection with anti-CD138 magnetic activated cell separation micro beads (Miltenyi Biotech). Tumor cells were purified from the BM of patients with MM using the RosetteSep negative selection system (StemCell Technologies). RosetteSep antibody cocktail is added to BM samples, and CD138-negative cells are crosslinked to RBC (rosetted) with RosetteSep reagents, followed by incubation for 20 min at room temperature and separation by Ficoll density centrifugation, as described previously (13).

Immunofluorescence. Cells grown on glass coverslips were fixed in cold absolute acetone for 10 min. After fixation, cells were washed in PBS and then blocked for 60 min with 5% fetal bovine serum in PBS. Slides were then incubated with anti-CD138 antibody (Santa Cruz Biotechnology) for 24 h at 4°C, washed in PBS, and incubated with FITC-conjugated goat anti-mouse IgG for 1 h at 4°C. Slides were analyzed using Nikon E800 fluorescence microscopy, as previously described (14, 15).

Growth inhibition assay and proliferation assay. The growth inhibitory effect of nBT062-SMCC-DM1, nBT062-SPDB-DM4, and BT062-SPP-DM1 and dexamethasone on growth of MM cell lines, PBMCs, and BM stromal cells (BMSC) was assessed by measuring 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (Chemicon International) dye absorbance.
as previously described (16). One antibody molecule has attached to it ~3.5 molecules DM4. The molecular weight of the antibody is not significantly increased by the attachment of the DM4 molecules.

The ability for nBT062-SPDB-DM4 to mediate antigen-dependent bystander killing of proximal CD138-negative cells was evaluated. CD138-positive MM OPM2 cells (1 × 10⁶ per well) and CD138-negative Namalwa cells (3 × 10⁵ per well) were plated separately or mixed in 96-well round-bottomed plates and exposed to nBT062-SPDB-DM4 for 24 h. Cell viability was then assessed using WST-8 reagent. To evaluate growth inhibitory effects of immunocojugates against MM cells in the BM milieu, MM cells (2 × 10⁶ per well) were cultured for 48 h in BMSC (1 × 10⁶ per well) coated 96-well plates (Costar) in the presence or absence of the drugs. DNA synthesis was measured by [³H]thymidine uptake, with [³H]thymidine (0.5 μCi/well) added during the last 8 h of 48-h cultures. All experiments were done in quadruplicate.

Cell cycle analysis. MM cells (1 × 10⁶) were incubated with or without agents, washed with PBS, permeabilized by a 30-min exposure to 70% ethanol at −20°C, incubated with propidium iodide (50 μg/mL) in 0.5 mL PBS containing 20 units/mL RNase A (Roche Diagnostics) for 30 min at room temperature, and analyzed for DNA content by using flow cytometry.

Detection of apoptotic cells and caspase inhibitor. MM cells (1 × 10⁶) were incubated with or without agents, washed with PBS, stained with PE-conjugated Apo 2.7 antibody (7A6, Beckman Coulter, Inc.), and analyzed on RXP Cytomics software on an Epics flow cytometer (Beckman Coulter, Inc.). Benzoylcarbonyl-Val-Ala-Asp-fluoromethylketone (z-VAD-fmk; Bachem Bioscience, Inc.), a pan-caspase inhibitor, was added in culture medium to reach a final concentration of 50 μmol/L. OPM1 cells were treated with or without z-VAD-fmk for 1 h before drug treatment.

In vivo human MM MOLP-8 xenograft model. Severe combined immunodeficient (SCID) mice were inoculated s.c. with MOLP-8 cells (1 × 10⁶/mouse) in a mixture of serum-free media and Matrigel. Mice were randomized when tumors reached ~100 mm³ and treated by bolus i.v. injection, as indicated. Mouse body weight was monitored as a sign of toxicity, and tumors were measured twice weekly in three dimensions using a caliper. The tumor volume was measured in mm³ using the formula $V = \text{length} \times \text{width} \times \text{height} \times 1/2$. Mice were sacrificed when the tumors reached ~2,000 mm³ or if the tumor became necrotic.

Green fluorescent protein–positive human MM xenograft mouse model and SCID-hu mouse model. OPM1 cells were transfected with green fluorescent protein (OPM1-GFP) using a lentiviral vector, as previously described (17). CB17 SCID mice (48-54 days old) were purchased from Charles River Laboratories. All animal studies were conducted according to protocols approved by the Animal Ethics Committee of the Dana-Farber Cancer Institute. Mice were inoculated s.c. with 5 × 10⁶ OPM1-GFP MM cells in 100 μL RPMI 1640. When tumors became palpable, mice were assigned to the treatment group receiving 200 μg conjugate per mouse via tail vein injection weekly to estimate the tumor volume using the following formula representing the three-dimensional volume of an ellipse: $4/3 \times (\text{width} / 2)^2 \times (\text{length} / 2)$. Animals were sacrificed when tumors reached 2 cm or when moribund. Survival was evaluated from the first day of treatment until death. Tumor growth was evaluated using caliper measurements from the first day of treatment until day of sacrifice, day 10 for control, and day 21 for the nBT062-SPDB-DM4 treatment group. Mice were monitored by whole-body fluorescence imaging using Illumina Bright Light System LT-9900 (Lights Tool Research) after shaving the tumor area. The images were captured with a Canon IXY digital 700 camera. Ex vivo analysis of tumor image was captured with a LEICA DM IL microscope connected to the LEICA DFC300 FX camera at 40 units/0.60 (Leica).

Human fetal long bones were implanted into CB17 SCID mice (SCID-hu), as previously described (18). Briefly, 4 wk after bone implantation, 2.5 × 10⁶ INA-6 cells in a final volume of 100 μL of RPMI 1640 were injected directly into the human BM cavity in the SCID-hu mice. An increase in the levels of soluble human IL-6 receptor (shull-6R), which is released by INA-6 cells, was used as a parameter of MM cell growth and burden of disease in SCID-hu mice. Mice developed measurable serum shull-6R ~4 wk after INA-6 cell injection and then received 0.176 mg conjugate or vehicle control via tail vein injection weekly for 7 wk. After treatments, blood samples were collected and assayed for shull-6R levels by an ELISA (R&D Systems).

Flow cytometry. For CD138 staining, tumor cells were harvested, stained with anti-CD138-PE (Sigma Chemical), and analyzed using an Epics flow cytometer (Coulter Immunology).

Western blotting. MM cells were cultured with or without nBT062-SPDB-DM4, nBT062-SPDM1, or nBT062-SPP-DM1, harvested, washed, and lysed using radioimmunoprecipitation assay buffer containing 2 mmol/L Na₂VO₄, 5 mmol/L NaF, 1 mmol/L phenylmethylsulfonyl fluoride, and 5 mg/mL complete protease inhibitor, as described previously (19, 20). Whole-cell lysates (20-40 μg/lane) were subjected to SDS-PAGE, transferred to pure nitrocellulose membranes (Bio-Rad Laboratories), and immunoblotted with antibodies against poly(ADP-ribose) polymerase (PARP), caspase-8, caspase-3, and caspase-9 (Cell Signaling Technology), as well as α-tubulin and CD138 (Santa Cruz Biotechnology).

Soluble CD138 ELISA. Supernatants from the cell cultures and BM plasma from MM patients was assayed for soluble (s)-CD138 using a solid phase sandwich ELISA kit (Cell Science) according to manufacturer’s instructions. Samples were measured in triplicate, and assay range is 8 to 256 ng/mL.

Cell adhesion assay. BMSCs (1 × 10⁶/well) were seeded in 96-well plates and cultured overnight at 37°C. On the following day, MM cells were washed thrice with PBS and resuspended in serum-free RPMI. Cells (2 × 10⁶) in 100 μL media, with or without immunoconjugate, were added to each well. Each sample was run in triplicate. After 2 h of culture at 37°C, floating cells were removed by manual pipetting. The remaining adherent cells were cultured with 10% fetal bovine serum RPMI and pulsed with [³H]thymidine added (0.5 μCi/well) for the last 8 h to measure DNA synthesis.

Statistical analysis. The statistical significance of differences observed in drug-treated versus control cultures was determined using
Dunn’s multiple comparison tests. The minimal level of significance was $P < 0.05$. For in vivo experiments, tumor volumes were compared using one-way analysis of Dunn’s multiple comparison tests. Survival was assessed using Kaplan-Meier curves and log-rank analysis.

**Results**

**Expression of CD138 in MM cell lines.** We first evaluated the expression of CD138 in MM1S, OPM1, OPM2, RPMI8226, DOX40, MM1R, LR5, U266, MOLP-8, and INA-6 MM cell lines. Western blot analysis (Supplementary Fig. S1A) and flow cytometric analysis (Supplementary Fig. S1B) showed that CD138 is expressed on all MM cell lines tested except LR5 and DOX40. Immunofluorescence analysis (Supplementary Fig. S2) showed weak expression of CD138 on MM.1S, DOX40, and LR5 cells. All other cell lines showed high CD138 expression.

**Selective cytotoxicity of nBT062-maytansinoid conjugate against CD138-positive MM cell lines in vitro.** The in vitro cytotoxicity of the anti-CD138 antibody nBT062 conjugated with maytansinoids DM1 and DM4 was next evaluated. The nBT062 conjugates tested varied in the chemical linker used to attach the maytansinoid molecule to the antibody. The chemical structures of nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPP-DM1 are shown in Fig. 1. nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPP-DM1 (3-354 ng/mL) showed cytotoxicity against OPM1 and RPMI8226 cells (CD138 positive) in a dose-dependent fashion. In contrast, minimal cytotoxicity was noted in low CD138 expression cell lines (Fig. 2A; Supplementary Fig. S3A and B). We also examined the cytotoxicity of free toxin DM4 and naked BT062 antibody compared with nBT062-SPDB-DM4. nBT062-SPDB-DM4 induced significantly greater cytotoxicity against OPM1 cells than free DM4 ($P < 0.05$) Moreover, naked BT062 antibody is not cytotoxic against OPM1 cells (Fig. 2B).

Importantly, these agents were also cytotoxic against primary tumor cells from MM patient isolated by negative selection,
with IC\textsubscript{50} values of \textasciitilde 1 nmol/L (111-442 ng/mL) at 48 h (Fig. 2C). However, no cytotoxicity was observed against primary tumor cells from MM patients isolated by CD138-positive selection (Supplementary Fig. 4A) suggesting that CD138-binding is important for immunoconjugate-mediated cytotoxicity and that binding is blocked by the anti-CD138 antibody used in the positive selection procedure. Indeed, nonconjugated nBT062 completely abrogated cytotoxicity induced by nBT062-SPDB-DM4 in OPM2 cells (Supplementary Fig. S4B). Importantly, the maytansinoid conjugates did not induce cytotoxicity in PBMCs from healthy volunteers at concentrations as high as 12 nmol/L (1770 ng/mL), further showing the specificity of these agents for CD138-positive cells (Fig. 2D).

CD138-specific immunoconjugates induce cell cycle arrest, followed by caspase and PARP cleavage in OPM1 cells. Maytansinoids are antimitotic agents that inhibit tubulin polymerization and microtubule assembly, and the maytansinoids DM1 and DM4 induce growth arrest in tumor cells in the G\textsubscript{2}-M phase of the cell cycle (21). We, therefore, next examined the cell cycle profile of OPM1 cells after nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPP-DM1 treatment. As shown in Fig. 3A and Supplementary Fig. S5, treatment of OPM1 cells with 1,000 ng/mL of these three agents for 24 hours induced a time-dependent increase in G\textsubscript{2}-M phase cells. nBT062-SPDB-DM4 had the most potent effect. To determine whether the cytotoxicity induced by these agents is via an apoptotic mechanism, we carried out Apo 2.7 staining and assessed cleavage of caspases and PARP. Treatment of OPM1 cells with the maytansinoids significantly increased Apo 2.7-positive cells in a time-dependent fashion (Fig. 3B), associated with induction of cleavage of caspase-8, caspase-9, caspase-3, and PARP (Fig. 3C). Conversely, the pan-caspase inhibitor z-VAD-fmk (50 \mu mol/L) blocked nBT062-SPDB-DM4–induced caspase and PARP cleavage in OPM1 cells (Fig. 3D). These results indicate that cytotoxicity triggered by these maytansinoid conjugates is mediated via caspase-dependent (both intrinsic and extrinsic) apoptotic pathways.

The immunoconjugates overcome the protective effects of growth factors and BMSCs. Because IL-6 and insulin-like growth factor-1 (IGF-I) promote MM cell survival by inhibiting apoptosis, we next examined whether these immunoconjugates can overcome this protective effect. Neither IL-6 nor IGF-I was able to block the cytotoxicity induced by nBT062-SPDB-DM4 (Fig. 4A and B). We also examined the cytotoxicity triggered by the conjugates in the context of BMSCs. Although BMSCs significantly inhibited dexamethasone-induced growth inhibition, they were not able to protect against immunoconjugate-induced cytotoxicity in OPM1 cells (Fig. 4C).

nBT062-SPDB-DM4 and nBT062-SPP-DM1 inhibit adhesion of MM1S cells to BMSCs. Many studies have revealed that...
syndecan-1 (CD138) mediates interactions between cells and extracellular matrix proteins to function as an adhesion molecule (3, 4, 22). We, therefore, next evaluated whether these conjugates could inhibit MM cell adhesion to BMSCs. Pretreatment of BMSCs with nBT062-SPDB-DM4 had only a modest inhibitory effect on MM1S and OPM1 cell adhesion to BMSCs; however, pretreatment of MM1S cells with nBT062-SPDB-DM4 almost completely blocked MM1S cell adhesion to BMSCs, suggesting that CD138 mediates MM cell adhesion, which can be blocked by immunoconjugates (Fig. 4D).

Soluble CD138 levels are greater in MM cell culture supernatants than in BM plasma of MM patients. sCD138 can be cleaved by the action of secretases (23) and released from the cell surface, which may inhibit binding of anti-CD138 immunoconjugates to the MM cell surface. We, therefore, next measured sCD138 levels in MM cell culture supernatants and BM plasma from MM patients. As shown in Fig. 5, soluble CD138 concentrations in BM plasma from MM patients were lower than levels in culture supernatants from RPMI8226 and OPM1 MM cells. Because the immunoconjugates are cytotoxic against both RPMI8226 and OPM1 MM cells, these results suggest that levels of circulating sCD138 in BM plasma of MM patients will not inhibit binding of anti-CD138 immunoconjugate to MM cells.

nBT062-maytansinoid conjugates inhibit tumor growth in a human MM xenograft model and SCID-hu model. The in vivo efficacy of nBT062-SPDB-DM4, nBT062-SMCC-DM1, and nBT062-SPP-DM1 was next evaluated in SCID mice bearing established CD138-positive MOLP-8 human MM cells. A single i.v. administration of the immunoconjugates caused significant dose-dependent tumor growth inhibition and tumor regression at concentrations that were well tolerated, evidenced by stable body weight. nBT062-SPDB-DM4 was the most active conjugate tested in this model (Fig. 6A). In addition, weekly dosing of the nBT062-SMCC-DM1 (six doses of 13.8 μg/kg) completely blocked tumor growth during the dosing period (Supplementary Fig. S6A).

In a second study, the importance of antigen-targeting for the antitumor activity of nBT062-SPDB-DM4 and nBT062-SPP-DM1 was evaluated by comparing the activity of unconjugated maytansinoid DM4, native unmodified nBT062 antibody, and a nontargeting (irrelevant) huIgG1-SPDB-DM4 conjugate. Treatment with a single bolus i.v. injection of
nBT062-SPDB-DM4 and nBT062-SPP-DM1 (at a dose of ∼14 mg/kg) inhibited the growth of the MOLP-8 xenografts (Fig. 6B); nBT062-SPDB-DM4 was the most active conjugate. In contrast, minimal antitumor activity was observed with free DM4, nBT062 antibody, and the nonbinding DM4 conjugate, showing the importance of specific targeting by the nBT062-maytansinoid conjugates for their in vivo efficacy.

The efficacy of nBT062-SPDB-DM4 and nBT062-SPP-DM1 was also examined in mice bearing s.c. fluorescent OPM1 MM cells (OPM1 GFP+; Supplementary Fig. S7). Treatment of OPM1 MM tumor-bearing mice with nBT062-SPDB-DM4 (0.176 mg conjugate per mouse; ∼6 mg/kg) significantly inhibited MM tumor growth compared with control animals treated with control vehicles (Dunn’s multiple comparison test; control vehicle versus nBT062-SPDB-DM4 at 10 days after treatment, \( P < 0.01 \); Fig. 6C). Similar to the results observed in the MOLP-8 model, nBT062-SPP-DM1 was not as effective as nBT062-SPDB-DM4 (Dunn’s multiple comparison test; nBT062-SPP-DM1 versus nBT062-SPDB-DM4 at 10 days after treatment, \( P < 0.05 \)).

Ex vivo analysis of tumors excised from mice showed significantly increased apoptosis in the mice treated with nBT062-SPDB-DM4 versus control cohorts (Supplementary Fig. S9). Importantly, treatment with these agents did not affect body weight (Supplementary Fig. S10).

To examine the activity of nBT062-SPDB-DM4 and nBT062-SPP-DM1 on MM cell growth in the context of the human BM microenvironment in vivo, we next used a SCID-hu model, in which IL-6-dependent INA-6 cells are directly injected into a human bone chip implanted in SCID-mice. These SCID-hu mice bearing human bones engrafted with INA-6 cells were treated via tail vein with nBT062-SPDB-DM4, nBT062-SPP-DM1, or vehicle alone weekly for 7 weeks. The serum shull-6R levels released by INA-6 cells reflects tumor burden in this model. As shown in Fig. 6D, nBT062-SPDB-DM4 and nBT062-SPP-DM1 treatment caused significant inhibition of tumor growth compared with vehicle control.

Bystander killing. Antibody-maytansinoid conjugates similar to nBT062-SPDB-DM4 have been shown to be able to kill antigen-negative cells proximally to antigen-positive tumor cells (bystander killing; ref. 24). To determine whether nBT062-SPDB-DM4 mediates bystander killing, CD138-positive OPM2 cells and CD138-negative Namalwa cells, either cultured separately or together, were treated with nBT062-SPDB-DM4 for 120 hours. Whereas nBT062-SPDB-DM4 was inactive against CD138-negative Namalwa cells cultured alone, significant killing of the CD138-negative cells by nBT062-SPDB-DM4 was observed when cultured with CD138-positive OPM2 cells (Supplementary Fig. S11).

Discussion

CD138 is highly expressed on MM cells and is involved in their development and/or proliferation (2, 25, 26), making CD138 an attractive therapeutic target. CD138 may be a suitable target for an antibody-directed immunoconjugate, although the use of a murine antibody in prior studies has precluded their clinical development (27). In the current study, we have evaluated the antitumor activity of a series of immunoconjugates composed of the murine/human chimeric anti-CD138 antibody nBT062 conjugated with potent cytotoxic maytansinoid moieties. The immunoconjugates tested, nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPP-DM1, vary in the nature of the disulfide linkage that attaches the cytotoxic agent to the antibody.

A series of MM cell lines were tested for CD138 expression using flow cytometry and immunoblotting, and these cell lines were used for the evaluation of the activity of the nBT062 conjugates. The nBT062-maytansinoid conjugates were highly active...
against MM tumor cell lines and patient MM cells that expressed CD138, with nBT062-SPDB-DM4 being the most potent of the three conjugates tested. Importantly, little or no cytotoxicity was observed upon treatment of CD138-negative cell lines and PBMCs from healthy volunteers, suggesting that the immunoconjugates are selective for CD138-expressing cells.

In vivo studies with MM tumor xenografts in immunocompromised mice showed that nBT062-SPDB-DM4 is the most efficacious of the conjugates tested and that the antitumor activity in mice is dependent on specific targeting of the nBT062 conjugate. In vitro mechanistic studies also showed that nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPP-DM1 inhibited the proliferation of MM cells by inducing G2-M cell cycle arrest followed by apoptotic cell death, evidenced by dose-dependent cleavage of caspases and PARP, as well as increased APO2.7-positive cells. We and others have previously reported that IL-6 triggers proliferation of MM cells and promotes survival; however, neither IL-6 nor IGF-I protect against nBT062-SPDB-DM4-induced cytotoxicity, suggesting that these immunoconjugates can overcome the protective effects of these cytokines in the BM milieu.

We further evaluated the effect of the BM microenvironment on the antitumor activity of these immunoconjugates using MM cells cocultured with isolated BMSCs. Whereas coculture with BMSCs significantly inhibits the antiproliferative effects of dexamethasone, there was no effect on the cell killing activity of the nBT062-maytansinoid conjugates.

Previous studies have shown that IL-6 can bind to the soluble heparan sulfate side chain of proteoglycans, such as CD138 (syndecan-1). These heparan sulfate proteoglycans can function as coreceptors for the growth factors, thereby leading to increased cell growth, survival, and adhesion (31-33). Within...
the BM milieu, induction of IL-6 secretion from BMSCs is triggered by direct MM cell–BMSC contact mediated by adhesion molecules, such as integrins and CD44 on the surface of MM cells (15, 34, 35). Interestingly, nBT062-SPDB-DM4 and nBT062-SPB-DM1 can block the adhesion of MM cells to BMSCs, suggesting that the immunoconjugates may also function to overcome cell adhesion–mediated drug resistance to conventional therapies.

Our experiments suggest that free nBT062 can block the cytotoxicity of nBT062-SPDB-DM4, confirming selectivity. However, antibody-maytansinoid conjugates similar to nBT062-SPDB-DM4 can have potent cell killing effects not only on antigen-positive cells but also on antigen-negative cells in close proximity to the tumor cells. Importantly, the presence of antigen-positive cells is required for this so-called bystander killing. A general mechanism of cytotoxicity for disulfide bond-linked antibody-maytansinoid conjugates includes binding of the conjugate to target cells, internalization into the target cell, cleavage of the conjugate disulfide bond, and release of the maytansinoid moiety, which is then capable of killing the target and nearby non-target cells. We carried out studies that showed bystander killing of CD138-negative Namawla cells in the presence of CD138-positive OPM2 cells by nBT062-SPDB-DM4 (Supplementary Fig. S5). Bystander killing of target cells in close proximity to MM cells would be expected to (a) provide an advantage for the eradication of tumor cells that heterogeneously express CD138, such as the putative CD138-negative myeloma stem cell (36); (b) kill tumor stroma cells, thereby destroying the tumor microenvironment; and/or (c) prevent selection of BT062-resistant tumor cells.

The enzyme responsible for the shedding of CD138 (syndecan-1) from the cell surface has not been identified. The proteolytically released extracellular domain (ectodomain) of syndecan-1 retains its biologically active heparin sulfate chains. Therefore, shed sCD138 in the BM plasma of MM patients could interfere with the function of an nBT062-maytansinoid by blocking access to the surface of MM tumor cells or by increasing the plasma clearance of the conjugate. Importantly, we showed that the level of sCD138 in BM plasma of MM patients is less than in MM cell line supernatants, wherein the immunoconjugates show potent cell killing activity. Therefore, sCD138 levels in BM plasma of MM patients should not interfere with binding of immunoconjugates.

In summary, nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPB-DM1 have in vitro and in vivo antitumor activity against CD138-positive MM cells and can overcome the protective effects of cytokines and BMSCs. Our results provide the preclinical framework for clinical trials of the most potent of the immunoconjugates tested, nBT062-SPDB-DM4 (so-called BT062), to improve patient outcome in MM.

Disclosure of Potential Conflicts of Interest


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References


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