

The monoclonal antibody nBT062 conjugated to maytansinoids has potent and selective cytotoxicity against CD138 positive multiple myeloma cells in vitro and in vivo

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Abstract

CD138 (Syndecan1) is highly expressed on multiple myeloma (MM) cells. In this study, we examined the anti-MM effect of murine/human chimeric CD138-specific monoclonal antibody (mAb) nBT062 conjugated with highly cytotoxic maytansinoid derivatives in vitro and in vivo. These agents significantly inhibited growth of CD138-positive 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 PARP and caspase-3, -8 and -9. Non-conjugated nBT062 completely blocked cytotoxicity induced by nBT062-maytansinoid conjugate, confirming that binding is required for inducing cytotoxicity. Moreover, nBT062-maytansinoid conjugates blocked adhesion of MM cells to bone marrow stromal cells (BMSCs). Co-culture of MM cells with BMSCs, which protects against dexamethasone-induced death, had no impact on the cytotoxicity of the immunoconjugates. Importantly, nBT062-SPDB-DM4 and nBT062-SPP-DM1 significantly inhibited MM tumor growth in vivo in both human multiple myeloma xenograft mouse models and in SCID-human bone grafts (SCID-hu mouse model). These studies provide the preclinical framework supporting evaluation of nBT062-maytansinoid derivatives in clinical trials to improve patient outcome in MM.

Introduction

The cell surface proteoglycan CD138 (Syndecan1) is an integral membrane protein acting as a receptor for the extracellular matrix. Within the normal human hematopoietic compartment, CD138 is expressed exclusively on differentiated plasma cells and is a primary diagnostic marker of multiple myeloma (MM)¹. Several monoclonal antibodies (mAbs) (i.e., B-B4, BC/B-B4, B-B2, DL-101, 1 D4, MI15, 1.BB.210, 2Q1484, 5F7, 104-9, 281-2) specific for CD138 have been reported. B-B4, 1D4 and MI15 Abs, which bind to similar or closely-related epitopes, recognize both the intact CD138 molecule and the core protein (with the heparin sulphate chains removed), and target the same or closely related epitopes. B-B4 preferentially binds to membrane bound versus soluble CD138.² It is a murine IgG1 monoclonal antibody that binds to a linear epitope between residues 90-95 of the core protein

on human syndecan-1. Consistent with the expression pattern of CD138, B-B4 strongly reacts with the MM cell line RPMI 8226, but not with endothelial cells. Moreover, B-B4-saporin immunotoxin is highly cytotoxic to RPMI8226 cells.³

Here we demonstrate the anti-tumor efficacy of three novel anti-CD138 antibody-maytansinoid conjugates, nBT062-SMCC-DM1, nBT062-SPDB-DM4 and nBT062-SPP-DM1, which vary in the linkage between the maytansinoid moiety and mAb. The nBT062-SMCC-DM1 linkage contains a thioether bond which is not cleavable by disulfide exchange, whereas the nBT062-SPDB-DM4 and nBT062-SPP-DM1 conjugates contain disulfide linkages which can be cleaved by disulfide exchange, resulting in liberation of active maytansinoid agent. The anti-CD138 antibody nBT062 is a murine/human chimeric form of B-B4, with identical specificity for CD138 as the parent murine antibody. The observed preclinical anti-tumor activity of the nBT062-maytansinoid conjugates provides the framework for clinical development of these agents to improve patient outcome in MM.

Materials and Methods

Detailed information pertinent to tumor cell lines and primary tumor cells from MM patients; Growth inhibition assay and proliferation assay; cell cycle profiling; detection of apoptosis; immunoblotting; immunofluorescence: adhesion assay; sCD138-ELISA; human MM xenograft mouse model and SCID-hu mouse model are included as supplemental information on the Leukemia website. The statistical significance of differences observed in drug-treated versus control cultures was determined using Dunn's multiple comparison tests in vivo study. The minimal level of significance was $P < 0.05$. 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 (Supplemental Figure 1A), Immunofluorescence (Supplemental Figure 1B) and flow-cytometric analysis

(Fig.1) demonstrated that CD138 is expressed on all MM cell lines tested except LR5 and Dox40.

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 vary in the chemical linker used to attach the maytansinoid molecule to the Ab. The chemical structures of nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPP-DM1 are shown in Fig 2A. nBT062-SMCC-DM1, nBT062-SPDB-DM4, and nBT062-SPP-DM1 (3-354 ng/mL) demonstrated cytotoxicity against OPM1 and RPMI8226 cells (CD138-Positive) in a dose-dependent fashion. In contrast, minimal cytotoxicity was noted in CD138-negative cell lines (Fig.2B). Importantly, these agents were also cytotoxic against primary tumor cells from MM patient isolated by negative selection, with IC₅₀ values of approximately 1nM (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 (Supplemental Fig.2A) 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, non-conjugated nBT062 completely abrogated cytotoxicity induced by nBT062-SPDB-DM4 in OPM2 cells (Supplemental Fig.2B). Importantly, the maytansinoid conjugates did not induce cytotoxicity in PBMCs from healthy volunteers at concentrations as high as 12 nM (1770 ng/mL), further demonstrating 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 anti-mitotic agents that inhibit tubulin polymerization and microtubule assembly, and the maytansinoids DM1 and DM4 induce growth arrest tumor cells in the G₂/M phase of the cell cycle⁴. 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, treatment of OPM1 cells with 1000 ng/mL of these three agents for 24h

induced a time-dependent increase in G2/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, left and middle panel). Conversely, the pan-caspase inhibitor z-VAD-fmk (50 μ M) blocked nBT062-SPDB-DM4-induced caspase and PARP cleavage in OPM1 cells (Fig. 3C, right panel). 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 BMSC.

Since IL-6 and IGF-1 promote MM cell survival by inhibiting apoptosis, we next examined whether these immunoconjugates can overcome this protective effect. Neither IL-6 nor IGF-1 was able to block the cytotoxicity induced by these conjugates (Fig. 4A and B and data not shown). 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^{5-7 8}. 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 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 is blocked by immunoconjugates(Fig.4D).

Soluble CD138 levels are greater in MM cell culture supernatants than in MM patient BM plasma

sCD138 can be cleaved by the action of secretases⁹ 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 Figure 5, soluble CD138 concentrations in BM plasma from MM patients were lower than levels in culture supernatants from RPMI8226 and OPM1 MM cells. Since the immunoconjugates are cytotoxic against both RPMI8226 and OPM1 MM cells, these results suggest that levels of circulating sCD138 in MM patient BM plasma 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 intravenous 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 (Fig.6A). nBT062-SPDB-DM4 was the most active conjugate tested in this model. In addition, weekly dosing of the nBT062-SMCC-DM1 conjugate (6 doses of 13.8 mg/kg) completely blocked tumor growth during the dosing period. In a second study, the importance of antigen-targeting for the anti-tumor 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 non-targeting (irrelevant) hulgG1-SPDB-DM4 conjugate. Treatment with a single bolus IV injection of nBT062-SPDB-DM4 and nBT062-SPP-DM1 (at a dose of approximately 14 mg/kg) inhibited the growth of the MOLP-8 xenografts (Fig 6 B); nBT062-SPDB-DM4 was the most active conjugate. In contrast, minimal anti-tumor activity was observed with free DM4, nBT062 antibody, and the non-binding DM4 conjugate, demonstrating 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 subcutaneous fluorescent OPM1 MM cells (OPM1^{GFP+}) (Supplemental Fig.3). Treatment of OPM1 MM tumor-bearing mice with nBT062-SPDB-DM4 (0.176 mg conjugate

/mouse; approximately 6 mg/kg) significantly inhibited MM tumor growth compared with control animals treated with control vehicle (Dunn's multiple comparison test; control vehicle vs. nBT062-SPDB-DM4 at 10 days post-treatment: $P < 0.01$, Fig. 6C and Supplemental Fig.4A). 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 vs. nBT062-SPDB-DM4 at 10 days post-treatment: $P < 0.05$, Fig. 6B). Kaplan-Meier and log-rank analysis revealed a mean overall survival (OS) of 13.6 days in the control cohort (95% confidence interval [CI], 10-19 days) versus 26 days (95% CI, 23-42 days) in groups treated with nBT062-SPDB-DM4 (Supplemental Fig.4B). Ex vivo analysis of tumors excised from mice showed significantly increased apoptosis in the mice treated with nBT062-SPDB-DM4 versus control cohorts (Supplemental Fig.5) Importantly, Treatment with these agents did not affect body weight.

In order to examine the activity of nBT062-SPDB-DM4 and nBT062-SPP-DM1 on MM cells 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 sIL-6R levels released by INA-6 cells reflects tumor burden in this model. As shown in Fig. 6E, 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 proximal to antigen-positive tumor cells (bystander killing)¹⁰. To determine whether nBT062-SPDB-DM4 mediates bystander killing, CD138-positive OPM2 cells and CD138-negative Namalwa cells cultured separately or as a mixture were treated with nBT062-SPDB-DM4 for 120 hours. While 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 mixed with CD138-positive OPM2 cells. (Supplemental Fig.6)

Discussion

CD138 is highly expressed on MM cells and is involved their development and/or proliferation, 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 development¹¹. In the current study, we have evaluated the anti-tumor activity of a series of immunoconjugates comprised 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 peripheral blood mononuclear cells 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 anti-tumor activity in mice is dependent on specific-targeting of the nBT062 conjugate.

In vitro mechanistic studies also demonstrated 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 protects against dexamethasone-induced apoptosis via activation of PI3-K/Akt, MEK/ERK and JAK2/STAT3 signaling cascades¹²⁻¹⁴. IGF-I also promotes MM cell proliferation and 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 impact of the BM microenvironment on the anti-tumor activity of these immunoconjugates using MM cells co-cultured with isolated BMSCs. While co-culture with BMSCs significantly inhibits the anti-proliferative effects of dexamethasone, there was no impact on the cell killing activity of the nBT062-maytansinoid conjugates.

Previous studies have demonstrated that IL-6 can bind to the soluble heparin sulfate side chain of proteoglycans like CD138 (syndecan-1). These heparin sulfate proteoglycans can function as co-receptors for the growth factors, thereby leading to increased cell growth, survival, and adhesion¹⁵⁻¹⁷. 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^{8,18 19}. Interestingly, nBT062-SPDB-DM4 and nBT062-SPP-DM1 can block the adhesion of MM cells to BMSCs, suggesting that the immunoconjugates may also function to overcome cell adhesion-mediated drug resistance (CAM-DR) 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 the studies which demonstrated bystander killing of CD138-negative Namawla cells in the presence of CD138-positive OPM2 cells with nBT062-SPDB-DM4. (Supplemental Fig.5) Bystanders killing of non-target cells in close proximity to MM cells would be expected to 1) provide an advantage for the eradication of tumor cells that heterogeneously express CD138, such as the putative CD138 negative myeloma stem cells²⁰, 2) kill tumor stroma cells, thereby destroying the tumor

microenvironment and/or 3) 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 MM patient BM plasma is less than in MM cell line supernatants, where the immunoconjugates show potent cell killing activity. Therefore sCD138 levels in MM patient BM plasma should not interfere with binding of immunoconjugates.

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

Figure Legends

Figure 1

Expression of CD138 in MM cell lines: CD138 expression in MM cell lines was determined by flow cytometry.

Figure 2

nBT062-maytansinoid conjugates have selective cytotoxicity toward CD138-positive cell lines.(A) Structure of nBT062-maytansinoid conjugates. nBT062-SMCC-DM1 contains a thioether-linkage that is not a substrate for disulfide exchange reactions. nBT062-SPDB-DM4 and nBT062-SPP-DM1 contain hindered disulfide linkages. (B) CD138-positive OPM1 (■) and RPMI8226 (◆), and CD138-negative MM cell lines DOX40 (□) and MM1S (△) were treated with nBT062-SMCC-DM1, nBT062-SPDB-DM4 and nBT062-SPP-DM1 for the indicated time periods.(C) Primary tumor cells from MM patients were isolated by negative selection and cultured with nBT062-SMCC-DM1 (◇), nBT062-SPDB-DM4 (□), and

nBT062-SPP-DM1 (\triangle) for 48h. (D) PBMCs isolated from normal donors were cultured with nBT062-SMCC-DM1 (\diamond), nBT062-SPDB-DM4 (\square), nBT062-SPP-DM1 (\triangle) for 72h, with OPM1 cells (\blacksquare) serving as a positive control. Cell viability (B-D) was assessed by MTT assay, and the data shown represent mean \pm SD of triplicate cultures, expressed as percentage of untreated controls.

Figure3

nBT062-maytansinoid conjugates induce G2/M growth arrest, followed by caspase-dependent apoptosis. (A) Cell cycle analysis: OPM1 cells were treated with nBT062-maytansinoids for 0, 12, and 24h, and then subjected to propidium iodide (PI) staining. (B) OPM1 cells were cultured with nBT062-maytansinoid (885ng/mL) for 0 hours (\blacksquare), 24h (\blacksquare), 48h (\square) and 72h (\square). Apoptotic cells were assessed by Apo 2.7 staining using flow cytometric analysis. (C) OPM1 cells were cultured with nBT062-SPDB-DM4 (885ng/mL) for the indicated time periods (left panel). Cells were treated with increasing concentrations of nBT062-SPDB-DM4 (0-885ng/mL) for 48h (middle panel). OPM1 cells were pre-incubated with Z-VAD-fmk (50 μ mol/L) for 60 minutes prior to treatment with 24h nBT062-SPDB-DM4 (right panel). Total cell lysates were subjected to immunoblotting using anti-caspase-3, -8, -9, PARP, and α -tubulin Abs. FL indicates full-length protein and CL indicates cleaved protein, respectively.

Figure4

Effect of growth factors and BMSCs on the sensitivity of MM cells to nBT062-maytansinoid immunoconjugates OPM1 cells were cultured for 48h with control media (\blacksquare); or with nBT062-SPDB-DM4 at 55ng/ml (\blacksquare), 111ng/ml (\square), 221ng/ml (\square), in the presence or absence of (A) IL-6 (1 and 10ng/ml), (B) IGF-1(10 and 50 ng/mL) or (C) BMSCs. In BMSC co-culture, cells were incubated for 48h with control media (\blacksquare), and with 250nM (\blacksquare), 500nM (\square), 1000nM (\square) dexamethasone, in the presence or absence of BMSCs for 48h, as a positive control for drug resistance. DNA synthesis was determined by measuring [3H]-thymidine incorporation during the last 8 h of 72h cultures. Data represent means (\pm SD) of triplicate cultures. (D) MM1S cells and/or BMSCs were incubated with control media (\blacksquare),

nBT062-SMCC-DM1 (■), nBT062-SPDB-DM4 (■), and nBT062-SPP-DM1 (□) at 885ng/mL for 2 hours prior to adhesion. Adherent cells were assessed by measuring [3H]-thymidine (Perkin-Elmer, Boston, MA) uptake. Values represent the mean [3H]-thymidine incorporation (cpm) of triplicate cultures.

Figure5

Quantification of sCD138 levels in cell culture supernatants from MM cell lines and BM plasma of MM patients.

sCD138 levels in cell culture supernatants from MM1S, RPMI8226, OPM1, and DOX40 MM cell lines, as well as plasma from 15 MM patient BM, were measured by ELISA. Error bars indicate SD (\pm).

Figure6

In vivo efficacy of nBT062-maytansinoid conjugates against human MM xenografts in SCID mice. (A) Mice bearing established (approximately 100 mm³) MOLP-8 tumor xenografts were treated with a single IV administration at day 11 post-inoculation of PBS (■), or of nBT062-SPDB-DM4, nBT062-SPP-DM1, and nBT062-SMCC-DM1 at doses of 100 (▲), 250 (◆) and 450 (●) μ g/kg, expressed as linked maytansinoid. One group of mice was also treated with five weekly IV injections of nBT062-SMCC-DM1. (B) Mice bearing MOLP-8 xenografts were treated on Day 12 with a single IV dose of control vehicle (■) or with 250 μ g/kg (linked maytansinoid) of nBT062-SPP-DM1 (◆), nBT062-SPBD-DM4 (●) and hulgG-SPDB-DM4 (▼). Groups were also treated with a single injection of the free maytansinoid DM4 (X); at 250 μ g/kg and unmodified nBT062 antibody (▲) at 13.8 mg/kg, an antibody dose equivalent to the amount of antibody in the conjugate treatment groups). (C) Mice injected with 5×10^6 OPM1^{GFP+} cells were treated with control vehicle (◆), nBT062-SPDB-DM4 (■) and nBT062-SPP-DM1 (▲). Mean tumor volume was calculated as in Material and Methods. Error bars represent SD (\pm). (D) SCID-hu mice engrafted with INA-6 cells in human bone chip were monitored for tumor growth by serial serum measurements of shuLL-6R as a measure of MM cell growth. Mice were treated with nBT062-SPDB-DM4 (■) nBT062-SPP-DM1 (▲) or vehicle (◆) and the shuLL-6R

levels were determined every week.

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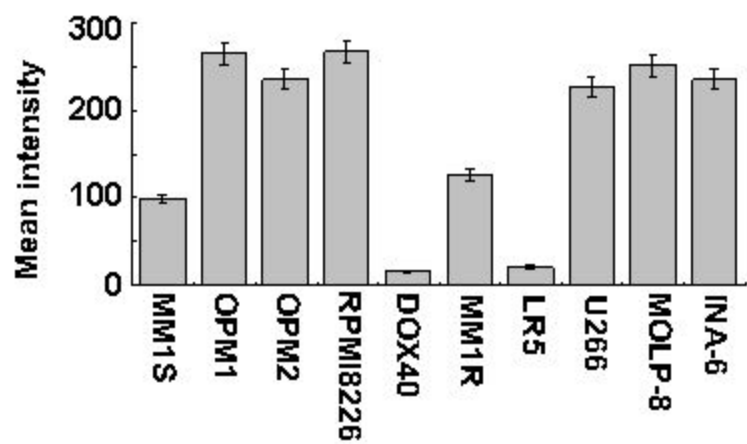


Figure.1

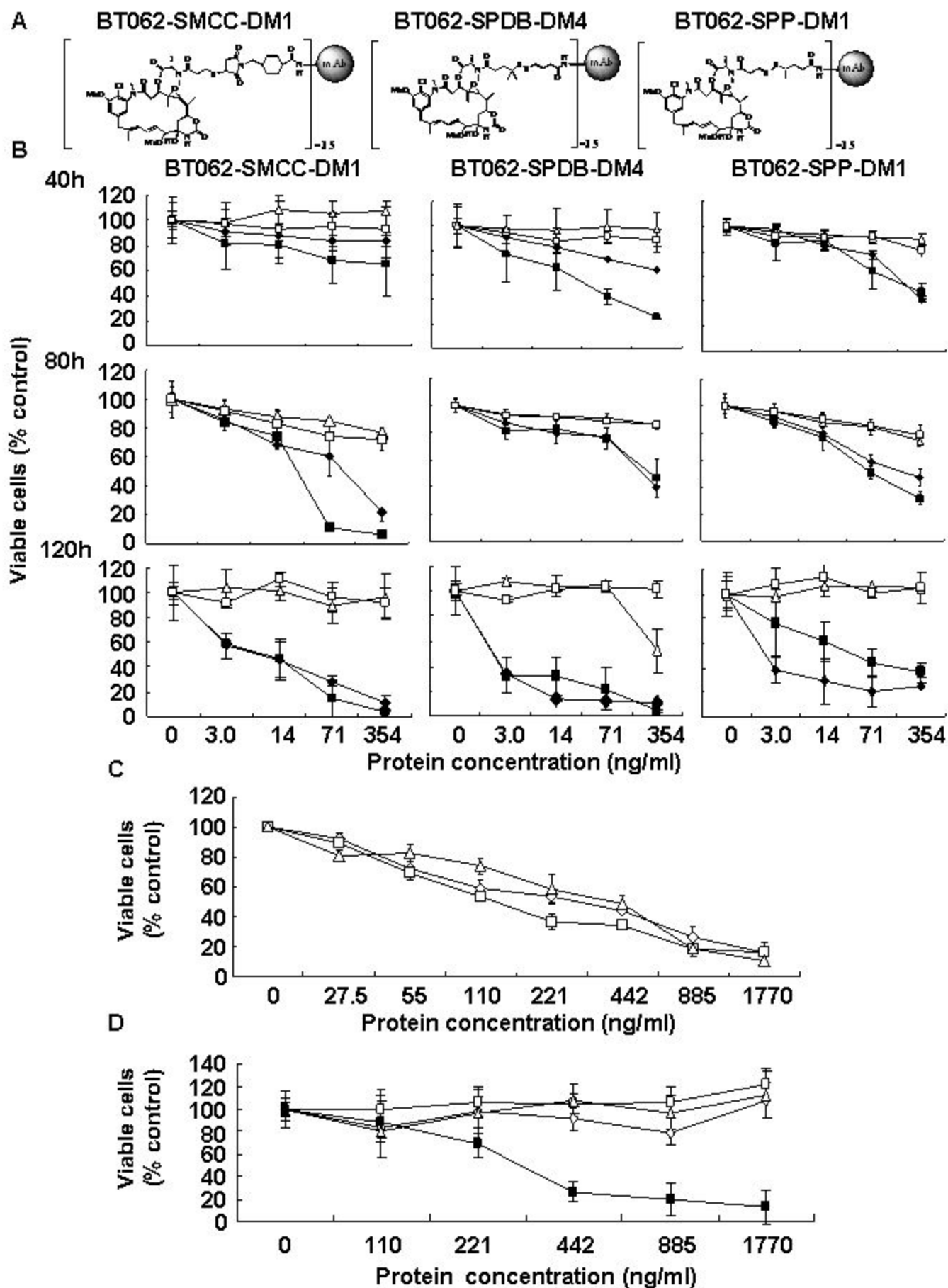


Figure.2

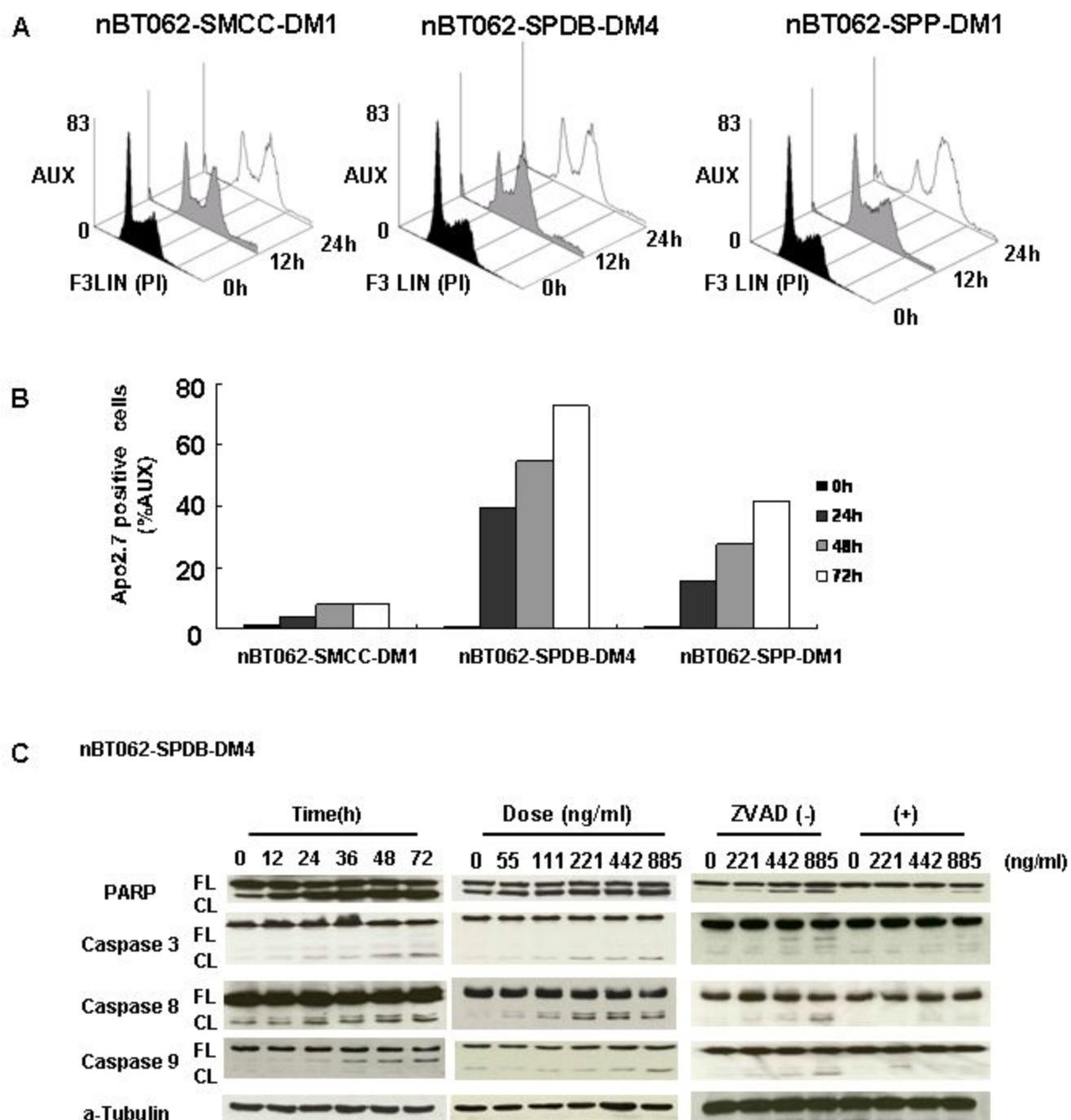


Figure.3

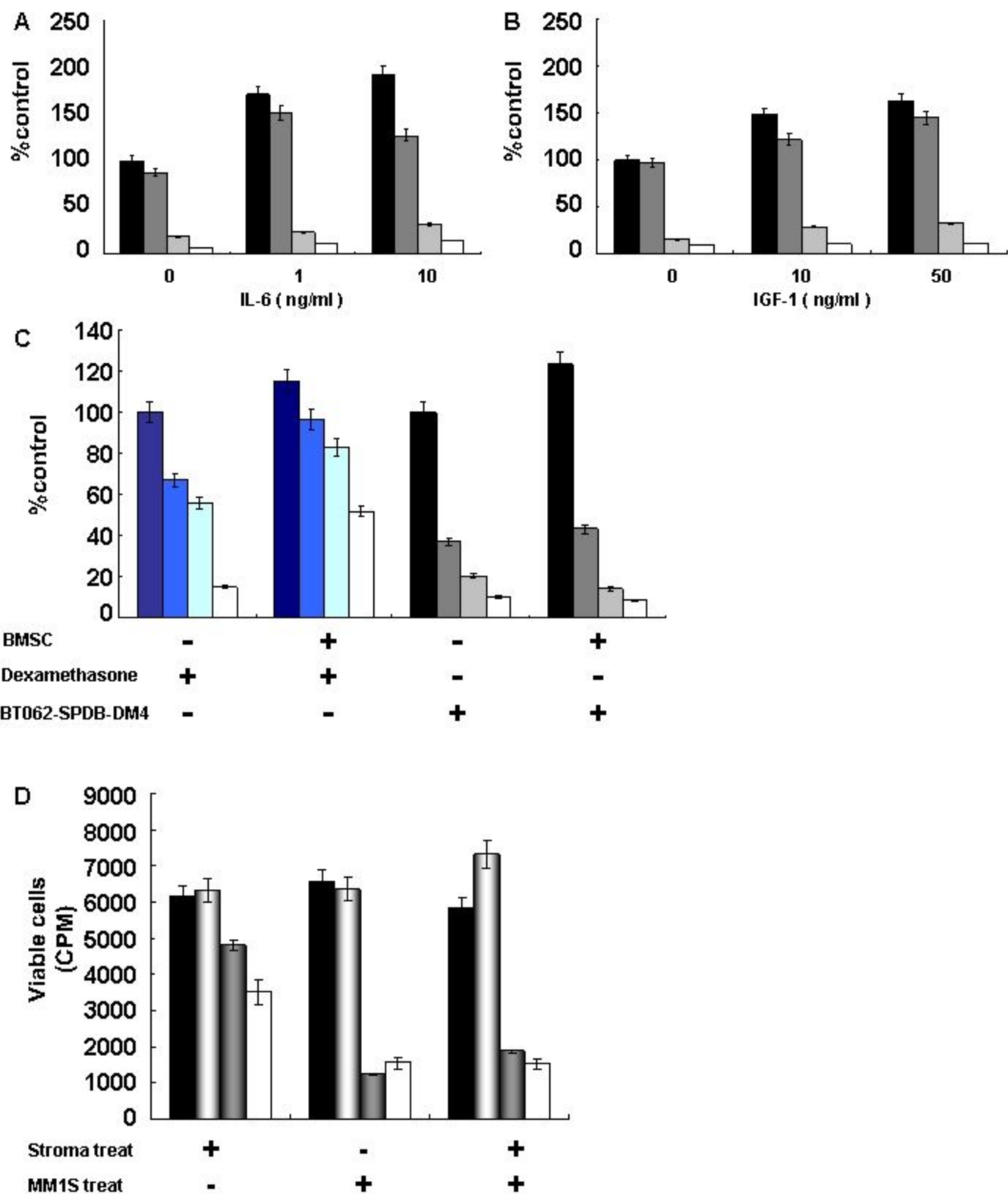


Figure.4

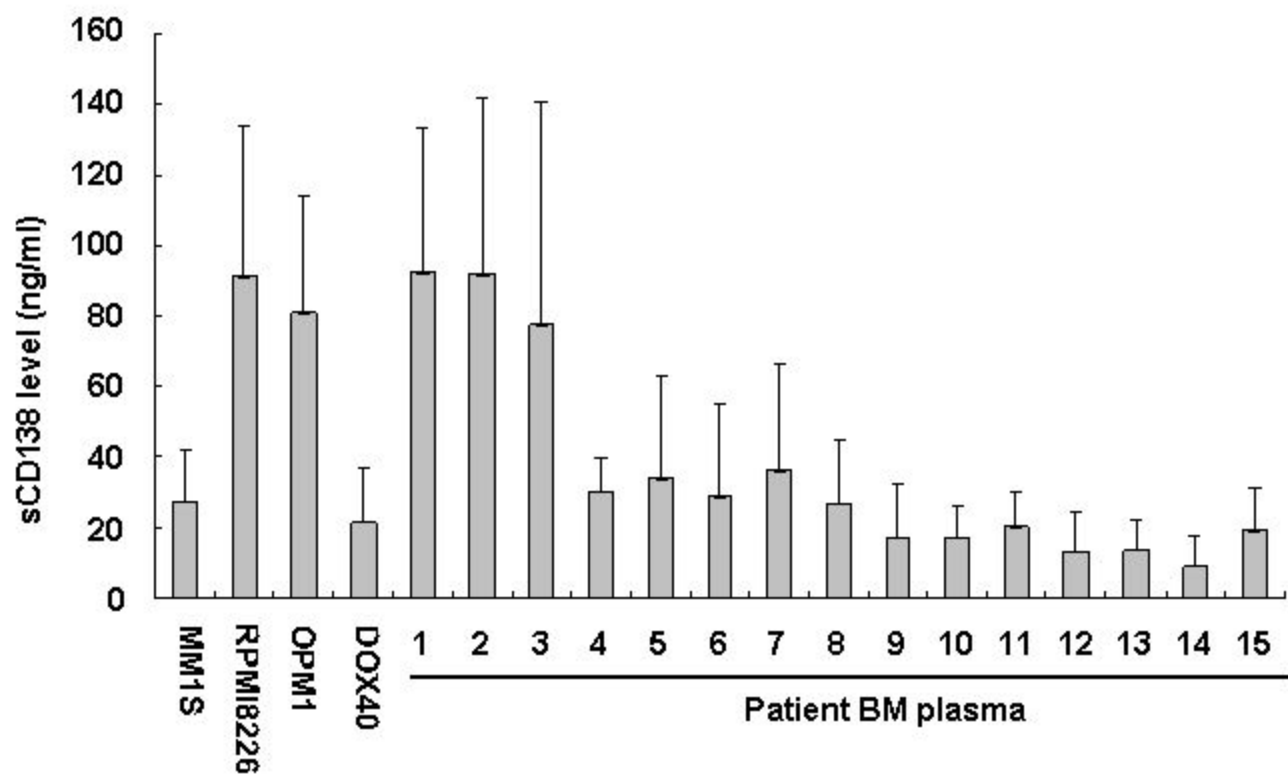


Figure.5

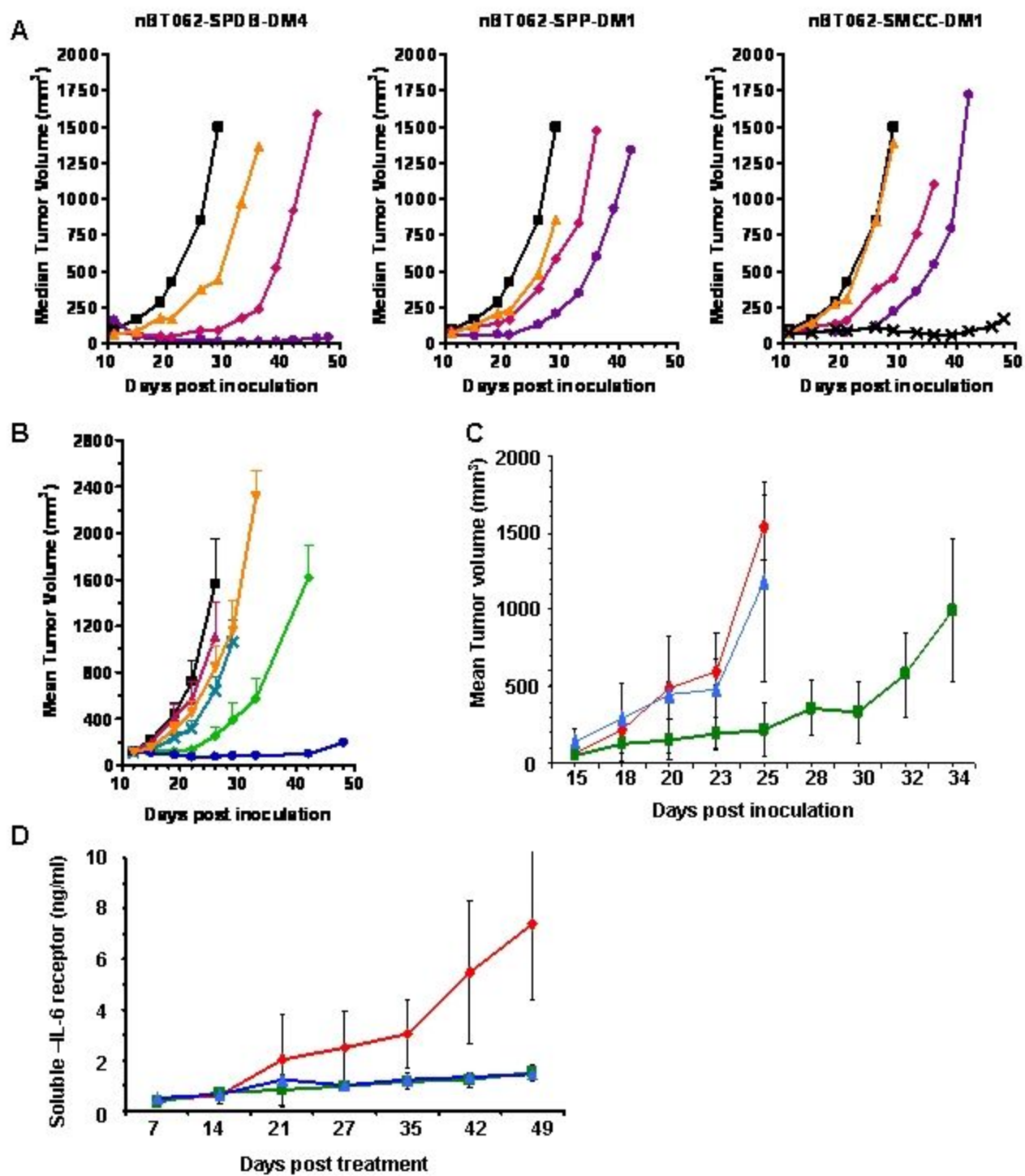


Figure.6