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

Hiroshi Ikeda,1,2 Teru Hideshima,3 Mariteresa Fulciniti,3 Robert J. Lutz,3 Tanyel Kiziltepe,3 Sonia Valle1, Samantha Pozzi,2 Loredana Sant2, Giulia Perrone,2 Yu-Tzu Tai,1 Diana Cristea,1 Noopur S. Raje,1 Christoph Uhlerk,1 Benjamin Dailen,1 Silke Aigner, Frank Osterre1, Nikhil Munshi,1 Paul Richardson1 and Kenneth C. Anderson1.

1 The Lebow Institute for Myeloma Therapeutics and Jerome Lipper Multiple Myeloma Center, Dana-Farber Cancer Institute Harvard Medical School, Boston, Massachusetts USA.
2 First Department of Internal Medicine, Sapporo Medical University, Sapporo, Japan.
3 ImmunoGen Inc., Waltham Massachusetts, USA.
4 Biocent AG, Landenheimerstra 6, Oeneich, Germany.

Purpose: We investigated the anti-tumor effect of murine/human chimeric CD138-specific monoclonal antibody (mAb) 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 nBT062-SPDB-DM4, BT062-SPDB-DM1, and BT062-SPP-DM1 against MM cell lines and primary tumor cells from MM patients in vitro. We also examined in vivo activity of these agents in a murine xenograft model transplanted with a human MM cell line. Anti-tumor activity was also analyzed in a SCID mouse model bearing implanted chips injected with a human MM cell line (SCID-hu model).

Results: Anti-CD138 immunconjugates significantly inhibited growth of MM cell lines and primary tumor cells from MM patients in vitro and in vivo, without cytotoxicity against peripheral blood mononuclear cells from healthy volunteers. In MM cells, the immunconjugates induced G2/M cell cycle arrest, followed by apoptosis associated with cleavage of caspases-3, -8, -9 and PARP. Non-conjugated 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 marrow stromal cells (BMSCs). Co-culture of MM cells with BMSCs protected against dexamethasone-induced death, but had no impact on the cytotoxicity of immunconjugates. 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 in the 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.

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Fig. 1: Structure of nBT062-maytansinoid conjugates. nBT062-SPDB-DM1 contains a threethreethreethreethreethreedot line. DM1 is not a substrate for disulfide exchange reactions. SPPDB is a mixture of two threethreethreethreethreedot lines. nBT062-STMP-DM1 contains threedot lines.

Fig. 2: nBT062-maytansinoid conjugates have selective cytotoxicity toward CD138-positive cell lines but not on human PBMCs. (A) Primary tumor cells from MM patients were selected by CD138 negative selection and cultured with nBT062-SPDB-DM1 (-), nBT062-SPDB-DM1 (■), nBT062-SPP-DM1 (△) or nBT062-SPP-DM1 (□) for 48 h. PBMCs isolated from normal donors were cultured with nBT062-SPDB-DM1 (-), nBT062-SPP-DM1 (■), nBT062-SPP-DM1 (△) or nBT062-SPP-DM1 (□) for 72h, with CD138+ OPM1 cells (■) serving as a positive control. Cell viability was assessed by MTT assay, and the data shown represent mean values &plusmn; 3D of duplicate cultures, expressed as percent survival of untreated cells.

Fig. 3: nBT062-maytansinoid conjugates induce G2/M growth arrest, followed by caspase-dependent apoptosis. CD138+ OPM1 cells were cultured with nBT062-SPDB-DM4 (885ng/mL) for the indicated time period. Total cell cultures were subjected to immunostaining using anti-caspase-3, -8, -9, PARP and a-lamin A. FL indicates full-length protein and CL indicates cleaved protein, respectively.

Fig. 4: Effect of growth factors and BMSCs on the sensitivity of MM cells to nBT062. MM1S treated with growth factors and BMSCs were treated with control media and with dexamethasone, in the presence or absence of BMSCs for 48h. Dexamethasone in the presence of BMSCs conferred drug resistance. DNA synthesis was determined by measuring [3H]thymidine incorporation during the last 6 h of 72h culture. Data represent growth of viable cultures. (A) MM1S cells and/or BMSCs were treated independently before culture with control media, nBT062-SPDB-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 uptake.

Fig. 5: In vivo efficacy of nBT062-SPDB-DM1 conjugates against human MM xenografts in SCID mice. (A) At day 11 post-inoculation mice bearing established CD138+ MM1S tumors xenografts were treated with a single administration of PBS (■), control (SPPDB or SPPDM) (△), or nBT062-SPDB-DM1 at different doses (■, 250mg/kg and 500mg/kg maytansinoid dose equivalent). (B) Data were fit to an exponential decay function, followed by determination of the half-life of the free maytansinoid DM1 (1.35h) and was adjusted for the maytansinoid dose equivalent to the amount of antibody used in the conjugate treatment groups. (C) Mice treated with 5.106 CFU CD138+ OPM1 cells were treated with control vehicle (■), nBT062-SPDB-DM1 (△), and nBT062-SPP-DM1 (□). Error bars represent SD (■). (D) SCID-hu mice injected with U-987 cells in human bone chips were monitored for tumor growth by serial serum measurements of shuIL-6 which correlates with tumor burden. Mice were treated with nBT062-SPDB-DM1 (■), nBT062-SPDB-DM4 (△) or vehicle (□) and shu-IL-6 levels were determined.