

## COMBINATION THERAPIES FOR HEME MALIGNANCIES WITH ANTI-CD38 ANTIBODIES AND SURVIVIN INHIBITORS

### FIELD OF THE INVENTION

The present invention relates to combination treatments for heme malignancies.

### BACKGROUND OF THE INVENTION

Multiple Myeloma (MM) is a B cell malignancy characterized by the latent accumulation of secretory plasma cells in bone marrow with a low proliferative index and an extended life span. The disease ultimately attacks bones and bone marrow, resulting in multiple tumors and lesions throughout the skeletal system. Approximately 1% of all cancers, and slightly more than 10% of all hematologic malignancies, can be attributed to MM. Incidence of MM increases in the aging population, with the median age at time of diagnosis being about 61 years.

Currently available therapies for MM include chemotherapy regimens, stem cell transplantation, THALOMID® (thalidomide), REVLIMID® (lenalidomide), POMALYST® (pomalidomide), VELCADE® (bortezomib), KYPROLIS® (carfilzomib), FARADYK® (panobinostat), AREDIA® (pamidronate), and ZOMETA® (zoledronic acid). Current treatment protocols, which include a combination of chemotherapeutic agents such as vincristine, BCNU, melphalan, cyclophosphamide, adriamycin, and prednisone or dexamethasone, yield a complete remission rate of only about 5%, and median survival is approximately 36-48 months from the time of diagnosis. Recent advances using high dose chemotherapy followed by autologous bone marrow or peripheral blood mononuclear cell transplantation have increased the complete remission rate and remission duration. Nevertheless, overall survival has only been slightly prolonged, and no evidence for a cure has been obtained. Ultimately, all MM patients relapse, even under maintenance therapy with interferon-alpha (IFN- $\alpha$ ) alone or in combination with steroids.

Efficacy of the available drug treatment regimens for MM is limited by the low cell proliferation rate and development of drug resistance in up to 90% of patients. Chromosomal translocations, oncogene mutations, dysregulated signaling pathways such as anti-apoptotic and survival pathways and bone marrow niche have been implicated to contribute to drug resistance in MM (for review, see Abdi *et al.*, *Oncotarget* 4:2186-2207, 2013). The bone marrow (BM) niche is implicated in proliferation, survival,

differentiation, migration, and drug resistance of the malignant plasma cells (Manier *et al.*, J Biomed Biotechnol 2012; published online 2012 October 3, doi: 10.1155/2012/157496).

CD38, a type II transmembrane glycoprotein is an attractive target for antibody therapeutics for various heme malignancies, including multiple myeloma. Anti-CD38 antibodies are described, for example, in Intl. Pat. Publ. No. WO2008/037257, Intl. Pat. Publ. No. WO2008/047242 and Intl. Pat. Publ. No. WO2007/042309, and are being evaluated in clinical settings for their efficacy in multiple myeloma and other heme malignancies.

#### SUMMARY OF THE INVENTION

The invention provides for a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody and a survivin inhibitor for a time sufficient to treat the CD38-positive hematological malignancy.

#### BRIEF DESCRIPTION OF THE DRAWINGS

**Figure 1A.** Bone Marrow stromal cells (BMSCs) mediate protection against multiple myeloma (MM) cell killing by ADCC induced by anti-CD38 antibody daratumumab in MM cell lines. Luciferase transduced CD38<sup>+</sup> UM9 MM cells were cultured in the presence or absence of healthy donor BMSCs (HD-BMSCs) for 16 hours prior to incubation with serial concentrations of daratumumab and HD-PBMCs at a PBMC:MM cell ratio of 30:1. MM cell viability was determined after 4 hours by bioluminescence imaging (BLI). Percent (%) ADCC was calculated relative to the cell viability without daratumumab. Error bars indicate the standard error of the mean (SEM) of triplicate measurements. The data are representative for 3 independent experiments. The differences between cultures with or without BMSCs were tested with an unpaired t test  $\ast = p < 0.05$ .

**Figure 1B.** Bone Marrow stromal cells (BMSCs) mediate protection against multiple myeloma (MM) cell killing by ADCC induced by anti-CD38 antibody daratumumab in MM cell lines. Luciferase transduced CD38<sup>+</sup> RPMI8226 MM cells were cultured in the presence or absence of HD-BMSCs for 16 hours prior to incubation with serial concentrations of daratumumab and HD-PBMCs at a PBMC:MM cell ratio of 30:1. MM cell viability was determined after 4 hours by BLI. % ADCC was calculated relative to the cell viability without daratumumab. Error bars indicate the SEM of triplicate

measurements. The data are representative for 3 independent experiments. The differences between cultures with or without BMSCs were tested with an unpaired t test  $^{*} = p < 0.05$ .

**Figure 2A.** BMSCs mediate protection against MM cell killing by ADCC induced by anti-CD38 antibody daratumumab in primary MM patient samples. Full Bone marrow aspirates obtained from MM patient 1 was cultured in the presence (white bars) or absence (black bars) of autologous bone marrow stromal cells and then treated with daratumumab at indicated concentrations. The autologous cells present in aspirate were used as effector cells. Since BM-MNCs already contain NK cells as effector cells, no additional effector cells were added. The viability of CD138<sup>+</sup> MM cells in the cultures was determined after 24 hours by flow cytometry. Error bars indicate the SEM of triplicate measurements. The differences between cultures with or without BMSCs were tested with an unpaired t test.  $^{*} = p < 0.05$ . Top panel: patient #1, Bottom panel: patient #2. BMSC: bone marrow stromal cell. ADCC: antibody-dependent cell cytotoxicity.

**Figure 2B.** BMSCs mediate protection against MM cell killing by ADCC induced by anti-CD38 antibody daratumumab in primary MM patient samples. Full Bone marrow aspirates obtained from MM patient 2 was cultured in the presence (white bars) or absence (black bars) of autologous bone marrow stromal cells and then treated with daratumumab at indicated concentrations. The autologous cells present in aspirate were used as effector cells. Since BM-MNCs already contain NK cells as effector cells, no additional effector cells were added. The viability of CD138<sup>+</sup> MM cells in the cultures was determined after 24 hours by flow cytometry. Error bars indicate the SEM of triplicate measurements. The differences between cultures with or without BMSCs were tested with an unpaired t test.  $^{*} = p < 0.05$ . Top panel: patient #1, Bottom panel: patient #2. BMSC: bone marrow stromal cell. ADCC: antibody-dependent cell cytotoxicity.

**Figure 3.** YM155 does not induce NK cell lysis. HD-PBMCs and patient bone marrow mononuclear cells (BMMNCs) were incubated with the indicated concentrations of YM155 for 24 hours. The number of viable CD3<sup>+</sup> CD56<sup>+</sup> NK cells was determined by flow cytometry and the percent lysis was calculated using the untreated samples as negative control.

**Figure 4A.** Daratumumab in combination with YM155 provide synergistic effect on MM cell killing by ADCC in the presence of stromal cells. Luciferase transduced RPMI8226 MM cells were cultured in presence or absence of HD-BMSCs. Daratumumab and YM155 were added at the indicated concentrations. HD-PBMCs were added in all wells

at a PBMC:MM ratio of 40:1 as a source of NK cells to induce ADCC. RPMI8226 cell survival was determined after 4 hours by BLI. The % lysis was calculated relative to the survival of RPMI8226 cells that did not receive any treatment. In the case of the YM155 and daratumumab combination, the expected lysis values were deduced from daratumumab and YM155 treatment alone, assuming that the cumulative effect would be additive, but not synergistic. The statistical differences between the expected and observed results were determined in a paired t test \*\*\* =  $P < 0.005$ , \* =  $P < 0.05$ . DARA: daratumumab.

**Figure 4B.** Daratumumab in combination with YM155 provides synergistic effect on primary MM cell killing by ADCC in the presence of stromal cells. Full BM aspirates of the MM patient 1 were cultured in the presence or absence of autologous MM-BMSCs. Daratumumab and YM155 were added at the indicated concentrations. Since BM-MNCs contain sufficient NK cells (in both cases, approximately at a 30:1 NK: MM cell ratio), no additional effector cells were added. After 24 hours, the viable CD138<sup>+</sup> MM cells were enumerated in each condition via flow cytometry. The % lysis was calculated relative to the survival of MM cells in BM-MNCs which were cultured at the same conditions but did not receive any treatment. The statistical differences between the expected and observed results were determined in a paired t test. \* =  $P < 0.05$ .

**Figure 4C.** Daratumumab in combination with YM155 provides synergistic effect on primary MM cell killing by ADCC in the presence of stromal cells. Full BM aspirates of the MM patient 2 were cultured in the presence or absence of autologous MM-BMSCs. Daratumumab and YM155 were added at the indicated concentrations. Since BM-MNCs contain sufficient NK cells (in both cases, approximately at a 30:1 NK: MM cell ratio), no additional effector cells were added. After 24 hours, the viable CD138<sup>+</sup> MM cells were enumerated in each condition via flow cytometry. The % lysis was calculated relative to the survival of MM cells in BM-MNCs which were cultured at the same conditions but did not receive any treatment. The statistical differences between the expected and observed results were determined in a paired t test. \* =  $P < 0.05$ .

**Figure 4D.** Daratumumab in combination with YM155 provides synergistic effect on primary MM cell killing by ADCC in the presence of stromal cells. Bone marrow mononuclear cells (BM-MNCs) from four MM patients containing CD138<sup>+</sup> MM cells (45%, 5.5%, 10.2%, 21.6% for patient 1-4, respectively) were cultured in the presence or

absence of autologous MM-BMSCs. Daratumumab (1 ng/ml) and appropriate sub maximal concentrations of YM155 (125, 62, 75 and 50 ng/ml for patient 1-4, respectively) were added. Since BM-MNCs contained sufficient NK cells (7.9%, 7.9%, 10.3% and 9.5% respectively), no additional effector cells were added. After 24 hours, the viable CD138<sup>+</sup> MM cells were enumerated in each condition via flow cytometry. The % lysis was calculated relative to the survival of MM cells in BM-MNCs which were cultured at the same conditions but did not receive any treatment. The statistical differences between the expected and observed results were determined in a paired t test. \* =  $P < 0.05$ . ns: not significant. DARA: daratumumab.

**Figure 5.** Antitumor effect of daratumumab and YM155 combination therapy. Analysis of tumor load per treatment group in RAG2<sup>-/-</sup>γc<sup>-/-</sup> mice implanted with UM9 cells. Hybrid scaffolds coated with human MSCs and loaded with luciferase transduced MM cell line UM9 were implanted subcutaneously in the back of RAG2<sup>-/-</sup>γc<sup>-/-</sup> mice (4 scaffolds per mice). Ten days after implantation, the growing tumors were visualized and quantified by BLI. Different groups of mice (n=4) were then either treated with vehicle control (control) or treated with daratumumab, YM155 or daratumumab plus YM155 as indicated. YM155 (or its vehicle, PBS) was delivered with subcutaneous infusion pumps at a rate of 1 mg/kg/d YM155 for 10 days. Each mouse, including those in the control group, received T cell depleted HD-PBMCs ( $5 \times 10^6$  cells) as a source of human NK cells to induce ADCC. Mice were monitored weekly by BLI. Results are expressed as the mean tumor load in each scaffold. The error bars represent the SEM. The statistical differences between mice treated with daratumumab and mice treated with daratumumab plus YM155 were calculated using the Mann-Whitney U-test.

\*\*\*  $P < 0.001$ .

#### DETAILED DESCRIPTION OF THE INVENTION

“CD38” refers to the human CD38 protein (synonyms: ADP-ribosyl cyclase 1, cADPr hydrolase 1, cyclic ADP-ribose hydrolase 1). Human CD38 has an amino acid sequence as shown in SEQ ID NO: 1. it is well known that CD38 is a single pass type II membrane protein with amino acid residues 1-21 representing the cytosolic domain, amino acid residues 22-42 representing the transmembrane domain, and residues 43-300 representing the extracellular domain of CD38.

The term “antibodies” as used herein is meant in a broad sense and includes immunoglobulin molecules including, monoclonal antibodies including murine, human,

human-adapted, humanized and chimeric monoclonal antibodies, antibody fragments, bispecific or multispecific antibodies, dimeric, tetrameric or multimeric antibodies, and single chain antibodies.

Immunoglobulins can be assigned to five major classes, namely IgA, IgD, IgE, IgG and IgM, depending on the heavy chain constant domain amino acid sequence. IgA and IgG are further sub-classified as the isotypes IgA1, IgA2, IgG1, IgG2, IgG3 and IgG4. Antibody light chains of any vertebrate species can be assigned to one of two clearly distinct types, namely kappa ( $\kappa$ ) and lambda ( $\lambda$ ), based on the amino acid sequences of their constant domains.

The term "antibody fragments" refers to a portion of an immunoglobulin molecule that retains the heavy chain and/or the light chain antigen binding site, such as heavy chain complementarity determining regions (HCDR) 1, 2 and 3, light chain complementarity determining regions (LCDR) 1, 2 and 3, a heavy chain variable region (VH), or a light chain variable region (VL). Antibody fragments include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)<sub>2</sub> fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a domain antibody (dAb) fragment (Ward *et al.*, Nature 341:544- 546, 1989), which consists of a VH domain. VH and VL domains can be engineered and linked together via a synthetic linker to form various types of single chain antibody designs where the VH/VL domains pair intramolecularly, or intermolecularly in those cases when the VH and VL domains are expressed by separate single chain antibody constructs, to form a monovalent antigen binding site, such as single chain Fv (scFv) or diabody; described for example in Intl. Pat. Publ. Nos. WO1998/44001, WO1988/01649, WO1994/13804, and WO1992/01047. These antibody fragments are obtained using well known techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are full length antibodies.

The phrase "isolated antibody" refers to an antibody or antibody fragment that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody specifically binding CD38 is substantially free of antibodies that specifically bind antigens other than human CD38). An isolated antibody that specifically binds CD38, however, can have cross-reactivity to other antigens, such as orthologs of human CD38, such as *Macaca fascicularis* (cynomolgus) CD38. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

An antibody variable region consists of a “framework” region interrupted by three “antigen binding sites”. The antigen binding sites are defined using various terms: (i) Complementarity Determining Regions (CDRs), three in the VH (HCDR1, HCDR2, HCDR3), and three in the VL (LCDR1, LCDR2, LCDR3) are based on sequence variability (Wu and Kabat J Exp Med 132:211-50, 1970; Kabat et al Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991). (ii) “Hypervariable regions”, “HVR”, or “HV”, three in the VH (H1, H2, H3) and three in the VL (L1, L2, L3) refer to the regions of an antibody variable domains which are hypervariable in structure as defined by Chothia and Lesk (Chothia and Lesk Mol Biol 196:901-17, 1987). Other terms include “IMGT-CDRs” (Lefranc *et al.*, Dev Comparat Immunol 27:55-77, 2003) and “Specificity Determining Residue Usage” (SDRU) (Almagro Mol Recognit 17:132-43, 2004). The International ImMunoGeneTics (IMGT) database (<http://www.imgt.org>) provides a standardized numbering and definition of antigen-binding sites. The correspondence between CDRs, HVs and IMGT delineations is described in Lefranc *et al.*, Dev Comparat Immunol 27:55-77, 2003.

“Chothia residues” as used herein are the antibody VL and VH residues numbered according to Al-Lazikani (Al-Lazikani *et al.*, J Mol Biol 273:927-48, 1997).

“Framework” or “framework sequences” are the remaining sequences of a variable region other than those defined to be antigen binding sites. Because the antigen binding sites can be defined by various terms as described above, the exact amino acid sequence of a framework depends on how the antigen-binding site was defined.

“Humanized antibody” refers to an antibody in which the antigen binding sites are derived from non-human species and the variable region frameworks are derived from human immunoglobulin sequences. Humanized antibodies may include substitutions in the framework regions so that the framework may not be an exact copy of expressed human immunoglobulin or germline gene sequences.

“Human antibody” refers to an antibody having heavy and light chain variable regions in which both the framework and the antigen binding sites are derived from sequences of human origin. If the antibody contains a constant region, the constant region also is derived from sequences of human origin.

A human antibody comprises heavy or light chain variable regions that are “derived from” sequences of human origin if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin or rearranged immunoglobulin genes. Such systems include human immunoglobulin gene libraries displayed on phage, and transgenic non-human animals such as mice carrying human

immunoglobulin loci as described herein. "Human antibody" may contain amino acid differences when compared to the human germline or rearranged immunoglobulin sequences due to for example naturally occurring somatic mutations or intentional introduction of substitutions in the framework or antigen binding sites. Typically, a "human antibody" is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical in amino acid sequence to an amino acid sequence encoded by a human germline or rearranged immunoglobulin gene. In some cases, a "human antibody" may contain consensus framework sequences derived from human framework sequence analyses, for example as described in Knappik *et al.*, J Mol Biol 296:57-86, 2000), or synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, as described in, for example, Shi *et al.*, J Mol Biol 397:385-96, 2010 and Intl. Pat. Publ. No. WO2009/085462). Antibodies in which antigen binding sites are derived from a non-human species are not included in the definition of "human antibody".

Isolated humanized antibodies may be synthetic. Human antibodies, while derived from human immunoglobulin sequences, may be generated using systems such as phage display incorporating synthetic CDRs and/or synthetic frameworks, or can be subjected to *in vitro* mutagenesis to improve antibody properties, resulting in antibodies that do not naturally exist within the human antibody germline repertoire *in vivo*.

"Recombinant antibody" includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal for human immunoglobulin genes or a hybridoma prepared therefrom (described further below), antibodies isolated from a host cell transformed to express the antibody, antibodies isolated from a recombinant, combinatorial antibody library, and antibodies prepared, expressed, created or isolated by any other means that involve splicing of human immunoglobulin gene sequences to other DNA sequences, or antibodies that are generated *in vitro* using Fab arm exchange.

"Monoclonal antibody" refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope, or in a case of a bispecific monoclonal antibody, a dual binding specificity to two distinct epitopes. "Monoclonal antibody" therefore refers to an antibody population with single amino acid composition in each heavy and each light chain, except for possible well known alterations such as removal of C-terminal lysine from the antibody heavy chain. Monoclonal antibodies may have heterogeneous glycosylation within the antibody population. Monoclonal antibody may

be monospecific or multispecific, or monovalent, bivalent or multivalent. A bispecific antibody is included in the term monoclonal antibody.

"Epitope" refers to a portion of an antigen to which an antibody specifically binds. Epitopes usually consist of chemically active (such as polar, non-polar or hydrophobic) surface groupings of moieties such as amino acids or polysaccharide side chains and can have specific three-dimensional structural characteristics, as well as specific charge characteristics. An epitope can be composed of contiguous and/or discontinuous amino acids that form a conformational spatial unit. For a discontinuous epitope, amino acids from differing portions of the linear sequence of the antigen come in close proximity in 3-dimensional space through the folding of the protein molecule.

"Variant" refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications for example, substitutions, insertions or deletions.

"In combination with" means that two or more therapeutics can be administered to a subject together in a mixture, concurrently as single agents or sequentially as single agents in any order.

"Treat" or "treatment" refers to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the development or spread of a tumor or tumor cells. Beneficial or desired clinical results include alleviation of symptoms, diminishment of extent of disease, stabilized (i.e., not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable and prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

"Therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of a therapeutic or a combination of therapeutics to elicit a desired response in the individual. Exemplary indicators of an effective therapeutic or combination of therapeutics that may decline or abate in association with resistance include, for example, improved well-being of the patient, reduction of a tumor burden, arrested or slowed growth of a tumor, and/or absence of metastasis of cancer cells to other locations in the body.

“Synergy”, “synergism” or “synergistic” mean more than the expected additive effect of a combination.

“Inhibits growth” (e.g., referring to cells, such as tumor cells) refers to a measurable decrease in the cell growth *in vitro* or *in vivo* when contacted with a therapeutic or a combination of therapeutics or drugs when compared to the growth of the same cells grown in appropriate control conditions well known to the skilled in the art. Inhibition of growth of a cell *in vitro* or *in vivo* may be at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%. Inhibition of cell growth can occur by a variety of mechanisms, for example by ADCC, apoptosis, necrosis, or by inhibition of cell proliferation.

“Subject” includes any human or nonhuman animal. “Nonhuman animal” includes all vertebrates, e.g., mammals and non-mammals, such as nonhuman primates, sheep, dogs, cats, horses, cows chickens, amphibians, reptiles, etc. The terms “subject” and “patient” are used interchangeably herein.

“Survivin” as used herein refers to the survivin protein having the amino acid sequence shown in SEQ ID NO: 22. Survivin is a member of the inhibitor of apoptosis (IAP) family. Survivin is a dual functional protein acting as an apoptosis inhibitor and cell cycle regulator. Overexpression of survivin is observed in human malignancies and positively correlates with poor prognosis, tumor recurrence, and therapeutic resistance (Liu *et al.*, Cancer Biol. Ther., 7:1053-1060, 2008; Mita *et al.*, Clin Cancer Res., 14:5000-5005, 2008).

SEQ ID NO: 22

MGAPTLPPAWQPFLKDHRISTFKNWPFLEGCACTPERMAEAGFIHCPTENEPDLA  
QCFFCFKELEGWEPDDDDPIEEHKKHSSGCAFLSVKKQFEELTLGEFLKLDREKAKN  
KIAKETNNKKKEFEETAEEKVRRRAIEQLAAMD

“Survivin inhibitor” refers to a molecule that inhibits, antagonizes, reduces or suppresses survivin activity; e.g., a molecule that inhibits the anti-apoptotic activity of survivin in a cell. Survivin inhibitor may inhibit the anti-apoptotic activity of survivin by about 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%. Survivin inhibitors may be a small molecule, peptide, a vaccine, a polynucleotide, DNA or RNA molecule.

The present invention is based, at least in part, on the finding that bone marrow stromal cells (BMSC) residing in the BM niche protect MM cells against antibody-induced ADCC at least in part by upregulating survivin, and that survivin inhibitors improve antibody-mediated ADCC of MM cells and abrogate ADCC resistance induced by

BMSCs. BMSCs have been shown to protect MM cells from cytotoxic T-lymphocyte (CTL)-dependent lysis via cell adhesion-mediated immune resistance, and survivin has been found to be upregulated in the lysis-resistant MM cells (de Haart *et al.*, Clin Cance Res 19:5591-5601, 2013).

The invention provides for a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody and a survivin inhibitor for a time sufficient to treat the CD38-positive hematological malignancy..

The invention also provides for a method of inhibiting growth or proliferation of multiple myeloma cells in a subject, comprising administering an anti-CD38 antibody and a survivin inhibitor to the subject in need thereof for a time sufficient to inhibit growth or proliferation of multiple myeloma cells.

“CD38-positive hematological malignancy” refers to a hematological malignancy characterized by the presence of tumor cells expressing CD38, including leukemias, lymphomas and myeloma. Exemplary such CD38-positive hematological malignancies are precursor B-cell lymphoblastic leukemia/lymphoma and B-cell non-Hodgkin's lymphoma, acute promyelocytic leukemia, acute lymphoblastic leukemia and mature B-cell neoplasms, such as B-cell chronic lymphocytic leukemia(CLL)/small lymphocytic lymphoma (SLL), B-cell acute lymphocytic leukemia, B-cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, mantle cell lymphoma (MCL), follicular lymphoma (FL), including low-grade, intermediate- grade and high-grade FL, cutaneous follicle center lymphoma, marginal zone B-cell lymphoma (MALT type, nodal and splenic type), hairy cell leukemia, diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), plasmacytoma, multiple myeloma (MM), plasma cell leukemia, post-transplant lymphoproliferative disorder, Waldenstrom's macroglobulinemia, plasma cell leukemias and anaplastic large-cell lymphoma (ALCL).

CD38 is expressed in a variety of malignant hematological diseases, including multiple myeloma, leukemias and lymphomas, such as B-cell chronic lymphocytic leukemia, T- and B-cell acute lymphocytic leukemia, Waldenstrom's macroglobulinemia, primary systemic amyloidosis, mantle-cell lymphoma, pro-lymphocytic/myelocytic leukemia, acute myeloid leukemia, chronic myeloid leukemia, follicular lymphoma, Burkitt's lymphoma, large granular lymphocytic (LGL) leukemia, NK-cell leukemia and plasma-cell leukemia. Expression of CD38 has been described on epithelial/endothelial cells of different origin, including glandular epithelium in prostate, islet cells in pancreas, ductal epithelium in glands, including parotid gland, bronchial epithelial cells, cells in testis and ovary and tumor

epithelium in colorectal adenocarcinoma. Other diseases, where CD38 expression could be involved include, e.g., broncho-epithelial carcinomas of the lung, breast cancer (evolving from malignant proliferation of epithelial lining in ducts and lobules of the breast), pancreatic tumors, evolving from the  $\beta$ -cells (insulinomas), tumors evolving from epithelium in the gut (e.g. adenocarcinoma and squamous cell carcinoma), carcinoma in the prostate gland, and seminomas in testis and ovarian cancers. In the central nervous system, neuroblastomas express CD38.

In some embodiments, the CD38-positive hematological malignancy is multiple myeloma (MM), acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL).

In some embodiments, the CD38-positive hematological malignancy is MM.

In some embodiments, the CD38-positive hematological malignancy is ALL.

In some embodiments, the CD38-positive hematological malignancy is NHL.

In some embodiments, the CD38-positive hematological malignancy is DLBCL.

In some embodiments, the CD38-positive hematological malignancy is BL.

In some embodiments, the CD38-positive hematological malignancy is FL.

In some embodiments, the CD38-positive hematological malignancy is MCL.

In some embodiments, the CD38-positive hematological malignancy is AML.

In some embodiments, the CD38-positive hematological malignancy is CLL.

In some embodiments, the CD38-positive hematological malignancy is a plasma cell disease.

In some embodiments, the plasma cell disease is light chain amyloidosis (AL), multiple myeloma (MM) or Waldenstrom's macroglobulinemia.

In some embodiments, the plasma cell disease is AL.

In some embodiments, the plasma cell disease is MM.

In some embodiments, the plasma cell disease is Waldenstrom's macroglobulinemia.

Examples of B-cell non-Hodgkin's lymphomas are lymphomatoid granulomatosis, primary effusion lymphoma, intravascular large B-cell lymphoma, mediastinal large B-cell lymphoma, heavy chain diseases (including  $\gamma$ ,  $\mu$ , and  $\alpha$  disease), lymphomas induced by therapy with immunosuppressive agents, such as cyclosporine-induced lymphoma, and methotrexate-induced lymphoma.

In one embodiment, the disorder involving cells expressing CD38 is Hodgkin's lymphoma.

Other examples of disorders involving cells expressing CD38 include malignancies derived from T and NK cells including mature T cell and NK cell neoplasms including T-cell prolymphocytic leukemia, T-cell large granular lymphocytic leukemia, aggressive NK cell leukemia, adult T-cell leukemia/lymphoma, extranodal NK/T cell lymphoma, nasal type, 78 enteropathy-type T-cell lymphoma, hepatosplenic T-cell lymphoma, subcutaneous panniculitis-like T-cell lymphoma, blastic NK cell lymphoma, Mycosis Fungoides/Sezary Syndrome, primary cutaneous CD30 positive T-cell lymphoproliferative disorders (primary cutaneous anaplastic large cell lymphoma C-ALCL, lymphomatoid papulosis, borderline lesions), angioimmunoblastic T-cell lymphoma, peripheral T-cell lymphoma unspecified, and anaplastic large cell lymphoma.

Examples of malignancies derived from myeloid cells include acute myeloid leukemia, including acute promyelocytic leukemia, and chronic myeloproliferative diseases, including chronic myeloid leukemia.

Any anti-CD38 antibody may be used in the methods of the invention. The variable regions of the anti-CD38 antibodies may be obtained from existing anti-CD38 antibodies and optionally cloned as full length antibodies using standard methods. Exemplary antibody variable regions binding CD38 that may be used are described for example in Intl. Pat. Publ. Nos. WO05/103083, WO06/125640, WO07/042309, WO08/047242, WO12/092612, WO06/099875, and WO11/154453A1.

An exemplary anti-CD38 antibody that may be used is DARZALEX<sup>TM</sup> (daratumumab). DARZALEX<sup>TM</sup> (daratumumab) comprises a heavy chain variable region (VH) and a light chain variable region (VL) amino acid sequences as shown in SEQ ID NO: 4 and 5, respectively, a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 amino acid sequences as shown in SEQ ID NOs: 6, 7 and 8, respectively, and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 amino acid sequences as shown in SEQ ID NOs: 9, 10 and 11, respectively, and is of IgG1/ $\kappa$  subtype. DARZALEX<sup>TM</sup> (daratumumab) heavy chain amino acid sequence is shown in SEQ ID NO: 12 and light chain amino acid sequence shown in SEQ ID NO: 13.

SEQ ID NO: 1

MANCEFSPVSGDKPCCRLSRRAQLCLGVSILVLILVVVLAVVVPRWRQQWSGPGT  
TKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCNITEEDYQPLM

KLGTQTVPCNKILLWSRIKDLAQFTQVQRDMFTLEDTLGYLADDLTWCGEFN  
TSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRKIFDK  
NSTFGSVEVHNLQPEKVQTLAWVIHGGREDSRDLCQDPTIKELESII SKRNIQFSC  
KNIYRPDKFLQCVKNPEDSSCTSEI

SEQ ID NO: 2  
SKRNIQFSCKNIYR

SEQ ID NO: 3  
EKVQTLAWVIHGG

SEQ ID NO: 4  
EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSA  
ISGSGGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYFCAKDK  
ILWFGEPVFDYWGQGTLVTVSS

SEQ ID NO: 5  
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD  
ASN RATGIPARFSGSGGTDFLTISLLEPEDFAVYYCQQR SNWPPTFGQ  
GTKVEIK

SEQ ID NO: 6  
SFAMS

SEQ ID NO: 7  
AISGSGGGTTYADSVKG

SEQ ID NO: 8  
DKILWFGEPVFDY

SEQ ID NO: 9  
RASQSVSSYLA

SEQ ID NO: 10  
DASN RAT

SEQ ID NO: 11

QQRSNWPPTF

SEQ ID NO: 12

EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSAISGSG  
GGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYFCAKDKILWFGPEPVF  
DYWGQGTLLTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN  
SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVKDKRV  
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE  
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN  
KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE  
WESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHN  
HYTQKSLSLSPGK

SEQ ID NO: 13

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAT  
GIPARFSGSGSGTDFTLTISSELPEDFAVYYCQQRSNWPPTFGQGTKVEIKRTVAAP  
SVFIFFPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS  
KDSTYLSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Another exemplary anti-CD38 antibody that may be used is mAb003 comprising the VH and VL sequences of SEQ ID NOs: 14 and 15, respectively and described in U.S. Pat. No. 7,829,693.

SEQ ID NO: 14

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGRVIPF  
LGIANSAQKFQGRVTITADKSTSTAY  
MDLSSLRSED TAVYYCARDIAALGPFDYWGQGTLLTVSSAS

SEQ ID NO: 15

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQS  
GVPSRFSGSGSGTDFTLTISLQP  
EDFATYYCQYNSYPRTFGQGTKVEIK

Another exemplary anti-CD38 antibody that may be used is mAb024 comprising the VH and VL sequences of SEQ ID NOs: 16 and 17, respectively and described in U.S. Pat. No. 7,829,693.

SEQ ID NO: 16

EVQLVQSGAEVKKPGESLKISCKGSGYSFSNYWIGWVRQMPGKGLEWMGHIYPH  
DSDARYSPSFQGGVTFSSADKSISTAY  
LQWSSLKASDTAMYYCARHVGWGSRYWYFDLWGRGTLTVTVSS

SEQ ID NO: 17

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAT  
GIPARFSGSGSGTDFTLTISSELP  
EDFAVYYCQQRSNWPPTFGQGTKVEIK

Another exemplary anti-CD38 antibody that may be used is MOR-202 (MOR-03087) comprising the VH and VL sequences of SEQ ID NOs: 18 and 19, respectively and described in US. Pat. No. 8,088,896.

SEQ ID NO: 18

QVQLVESGGGLVQPGGSLRLSCAASGFTSSYYMNWVRQAPGKGLEWVSGISGD  
PSNTYYADSVKGRFTISRDNKNTLY  
LQMNSLRAEDTAVYYCARDLPLVYTGFAYWGQGTLTVTVSS

SEQ ID NO: 19

DIELTQPPSVSVAPGQTARISCSGDNLRHYVYVWYQQKPGQAPVLVIYGDSKRPS  
GIPERFSGSNSGNTATLTISGTQAE  
DEADYYCQTYTGGASLVFGGGTKLTVLGQ

Another exemplary anti-CD38 antibody that may be used is isatuximab, comprising the VH and VL sequences of SEQ ID NOs: 20 and 21, respectively, described in U.S. Pat. No. 8,153,765. The VH and the VL of isatuximab may be expressed as IgG1/κ.

SEQ ID NO: 20

QVQLVQSGAEVAKPGTSVKLSCKASGYTFTDYWMQWVKQRPGQGLEWIGT  
IYPGDGDTGYAQKFQGKATLTADKSSKTVYMHLSLASEDSAVYYCARGD

YYGSNSLDYWGQGTSTVTVSS

SEQ ID NO: 21

DIVMTQSHLSMSTSLGDPVSITCKASQDVSTVVAWYQQKPGQSPRLIYS  
ASYRYIGVPDRFTGSGAGTDFTFITISSVQAEDLAVYYCQQHYSPPYTFGG  
GTKLEIK

Anti-CD38 antibodies used in the methods of the invention may also be selected *de novo* from for example a phage display library, where the phage is engineered to express human immunoglobulins or portions thereof such as Fabs, single chain antibodies (scFv), or unpaired or paired antibody variable regions (Knappik *et al.*, J Mol Biol 296:57-86, 2000; Krebs *et al.*, J Immunol Meth 254:67-84, 2001; Vaughan *et al.*, Nature Biotechnology 14:309-314, 1996; Sheets *et al.*, PITAS (USA) 95:6157-6162, 1998; Hoogenboom and Winter, J Mol Biol 227:381, 1991; Marks *et al.*, J Mol Biol 222:581, 1991). CD38 binding variable domains may be isolated for example from phage display libraries expressing antibody heavy and light chain variable regions as fusion proteins with bacteriophage pIX coat protein as described in Shi *et al* (2010) *J. Mol. Biol.* 397:385-96 and Intl. Pat. Publ. No. WO09/085462). The antibody libraries may be screened for binding to human CD38 extracellular domain and the obtained positive clones may be further characterized and the Fabs isolated from the clone lysates, and subsequently cloned as full length antibodies. Such phage display methods for isolating human antibodies are established in the art. See for example: US Pat. No. 5,223,409; US Pat. No. 5,403,484; and US Pat. No. 5,571,698, US Pat. No. 5,427,908, US Pat. No. 5, 580,717, US Pat. No. 5,969,108, US Pat. No. 6,172,197, US Pat. No. 5,885,793; US Pat. No. 6,521,404; US Pat. No. 6,544,731; US Pat. No. 6,555,313; US Pat. No. 6,582,915 and US Pat. No. 6,593,081.

The invention also provides for a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody that competes for binding to CD38 with an antibody comprising the VL of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 and a survivin inhibitor for a time sufficient to treat the CD38-positive hematological malignancy.

The invention also provides for a method of treating a subject having multiple myeloma, comprising administering to the subject in need thereof an anti-CD38 antibody that competes for binding to CD38 with an antibody comprising the VH of SEQ ID NO: 4

and the VL of SEQ ID NO: 5 and a survivin inhibitor for a time sufficient to treat multiple myeloma.

The invention also provides for a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody that binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) and a survivin inhibitor for a time sufficient to treat the CD38-positive hematological malignancy.

The invention also provides for a method of treating a subject having multiple myeloma, comprising administering to the subject in need thereof an anti-CD38 antibody that binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) and a survivin inhibitor for a time sufficient to treat multiple myeloma.

In some embodiments, the anti-CD38 antibody comprises the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 6, 7 and 8, respectively.

In some embodiments, the anti-CD38 antibody comprises the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 9, 10 and 11, respectively.

In some embodiments, the anti-CD38 antibody comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 6, 7, 8, 9, 10 and 11, respectively.

In some embodiments, the anti-CD38 antibody comprises the VL comprising an amino acid sequence that is 95%, 96%, 97%, 98%, 99% or 100% identical to that of SEQ ID NO: 4 and the VH comprising an amino acid sequence that is 95%, 96%, 97%, 98%, 99% or 100% identical to that of SEQ ID NO: 5.

In some embodiments, the anti-CD38 antibody comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5.

In some embodiments, the anti-CD38 antibody comprises the VH of SEQ ID NO: 14 and the VL of SEQ ID NO: 15.

In some embodiments, the anti-CD38 antibody comprises the VH of SEQ ID NO: 16 and the VL of SEQ ID NO: 17.

In some embodiments, the anti-CD38 antibody comprises the VH of SEQ ID NO: 18 and the VL of SEQ ID NO: 19.

In some embodiments, the anti-CD38 antibody comprises the VH of SEQ ID NO: 20 and the VL of SEQ ID NO: 21.

Antibodies may be evaluated for their competition with a reference antibody, for example DARZALEX™ (daratumumab having the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for binding to CD38 using well known *in vitro* methods. In an exemplary method, CHO cells recombinantly expressing CD38 may be incubated with unlabeled reference antibody for 15 min at 4°C, followed by incubation with an excess of fluorescently labeled test antibody for 45 min at 4°C. After washing in PBS/BSA, fluorescence may be measured by flow cytometry using standard methods. In another exemplary method, extracellular portion of human CD38 may be coated on the surface of an ELISA plate. Excess of unlabelled reference antibody may be added for about 15 minutes and subsequently biotinylated test antibodies may be added. After washes in PBS/Tween, binding of the test biotinylated antibody may be detected using horseradish peroxidase (HRP)-conjugated streptavidin and the signal detected using standard methods. It is readily apparent that in the competition assays, the reference antibody may be labeled and the test antibody unlabeled. The test antibody competes with the reference antibody when the reference antibody inhibits binding of the test antibody, or the test antibody inhibits binding of the reference antibody by at least 80%, 85%, 90%, 95% or 100%. The epitope of the test antibody may further be defined for example by peptide mapping or hydrogen/deuterium protection assays using known methods, or by crystal structure determination.

An anti-CD38 antibody binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) when the antibody binds at least 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 residues within SEQ ID NO: 2 and SEQ ID NO: 3. In some embodiments, the anti-CD38 antibody binds at least one amino acid in the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and at least one amino acid in the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1). In some embodiments, the anti-CD38 antibody binds at least two amino acids in the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and at least two amino acids in the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1). In some embodiments, the anti-CD38 antibody binds at least three amino acids in the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and at least three amino acids in the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1).

An exemplary antibody that binds to the region SKRNIQFSCCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) is DARZALEX™ (daratumumab).

Antibodies binding to the region SKRNIQFSCCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) may be generated for example by immunizing mice with peptides having the amino acid sequences shown in SEQ ID NOs: 2 and 3 using standard methods and as described herein, and characterizing the obtained antibodies for binding to the peptides using for example ELISA or mutagenesis studies.

The Fc portion of the antibody may mediate antibody effector functions such as antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) or complement dependent cytotoxicity (CDC), as described in more detail below. Such functions may be mediated by binding of an Fc effector domain(s) to an Fc receptor on an immune cell with phagocytic or lytic activity or by binding of an Fc effector domain(s) to components of the complement system. Typically, the effect(s) mediated by the Fc-binding cells or complement components result in inhibition and/or depletion of target cells, e.g., CD38-expressing cells. Human IgG isotypes IgG1, IgG2, IgG3 and IgG4 exhibit differential capacity for effector functions. ADCC may be mediated by IgG1 and IgG3, ADCP may be mediated by IgG1, IgG2, IgG3 and IgG4, and CDC may be mediated by IgG1 and IgG3.

In some embodiments, the anti-CD38 antibody is of IgG1, IgG2, IgG3 or IgG4 isotype.

In some embodiments, the anti-CD38 antibody is of IgG1 isotype.

In some embodiments, the anti-CD38 antibody is of IgG2 isotype.

In some embodiments, the anti-CD38 antibody is of IgG3 isotype.

In some embodiments, the anti-CD38 antibody is of IgG4 isotype.

In some embodiments, the anti-CD38 antibody induces killing of CD38-expressing cells by antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC) or apoptosis.

In some embodiments, the anti-CD38 antibody induces killing of CD38-expressing cells by ADCC.

In some embodiments, the anti-CD38 antibody induces killing of CD38-expressing cells by ADCP.

In some embodiments the anti-CD38 antibody induces killing of CD38-expressing cells by CDC.

In some embodiments, the anti-CD38 antibody induces killing of CD38-expressing cells by apoptosis.

"Antibody-dependent cellular cytotoxicity," "antibody-dependent cell-mediated cytotoxicity" or "ADCC" is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells with effector cells possessing lytic activity, such as natural killer cells, monocytes, macrophages and neutrophils via Fc gamma receptors (FcγR) expressed on effector cells. For example, NK cells express FcγRIIIa, whereas monocytes express FcγRI, FcγRII and FcγRIIIa. Death of the antibody-coated target cell, such as CD38-expressing MM cell, occurs as a result of effector cell activity through the secretion of membrane pore-forming proteins and proteases. To assess ADCC activity of an anti-CD38 antibody, the antibody may be added to CD38-expressing cells in combination with immune effector cells, which may be activated by the antigen antibody complexes resulting in cytolysis of the target cell. Cytolysis may be detected by the release of label (e.g., radioactive substrates, fluorescent dyes or natural intracellular proteins) from the lysed cells. Exemplary effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Multiple myeloma cell lines or primary MM cells that express CD38 may be used as target cells. In an exemplary assay, MM cell lines engineered to express luciferase are incubated with anti-CD38 antibodies. Freshly isolated PBMC effector cells are added at target:effector cell ratio of 40:1. 4 hours after addition of PBMC, luciferin is added and the resulting bioluminescent signal emitted from surviving MM cells determined within 20 minutes using a luminometer (SpectraMax, Molecular Devices), and the percentage ADCC of MM cells can be calculated using the formula: % ADCC = 1 - (mean bioluminescent signal in the absence of PBMCs / mean bioluminescent signal in the presence of PBMCs) x100%. Anti-CD38 antibody "induces ADCC *in vitro*" when % ADCC in an *in vitro* assay such as one described above is at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 80%, 90% or 100%.

"Complement-dependent cytotoxicity", or "CDC", refers to a mechanism for inducing cell death in which an Fc effector domain of a target-bound antibody binds and activates complement component C1q which in turn activates the complement cascade leading to target cell death. Activation of complement may also result in deposition of complement components on the target cell surface that facilitate ADCC by binding complement receptors (e.g., CR3) on leukocytes. In an exemplary assay, primary BM-MNC cells isolated from a patient with a B-cell malignancy may be treated with an anti-CD38 antibody and complement derived from 10% pooled human serum for 1 hour at a

concentration of 0.3-10 µg/ml, and the survival of primary CD138<sup>+</sup> MM cells may be determined by flow cytometry using techniques described in van der Veer *et al.*, Haematologica 96:284-290, 2011; van der Veer *et al.*, Blood Cancer J 1(10):e41, 2011. The percentage of MM cell lysis may be determined relative to an isotype control as described herein. Anti-CD38 antibodies used in the methods of the invention may induce CDC by about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

"Antibody-dependent cellular phagocytosis" ("ADCP") refers to a mechanism of elimination of antibody-coated target cells by internalization by phagocytic cells, such as macrophages or dendritic cells. ADCP may be evaluated by using monocyte-derived macrophages as effector cells and Daudi cells (ATCC<sup>®</sup> CCL-213<sup>™</sup>) or B cell leukemia or lymphoma tumor cells expressing CD38 as target cells engineered to express GFP or other labeled molecule. Effector:target cell ratio may be, for example, 4:1. Effector cells may be incubated with target cells for 4 hours with or without anti-CD38 antibody. After incubation, cells may be detached using accutase. Macrophages may be identified with anti-CD11b and anti-CD14 antibodies coupled to a fluorescent label, and percent phagocytosis may be determined based on % GFP fluorescent in the CD11<sup>+</sup>CD14<sup>+</sup> macrophages using standard methods. Anti-CD38 antibodies used in the methods of the invention may induce ADCP by about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100%.

ADCC elicited by anti-CD38 antibodies may be enhanced by certain substitutions in the antibody Fc. Exemplary substitutions are for example substitutions at amino acid positions 256, 290, 298, 312, 356, 330, 333, 334, 360, 378 or 430 (residue numbering according to the EU index) as described in U.S. Pat. No. 6,737,056.

In some embodiments, the anti-CD38 antibodies comprise an amino acid substitution in the antibody Fc.

In some embodiments, the anti-CD38 antibodies comprise a substitution in the antibody Fc at amino acid positions 256, 290, 298, 312, 356, 330, 333, 334, 360, 378 or 430 (residue numbering according to the EU index).

ADCC elicited by anti-CD38 antibodies can also be enhanced by engineering the antibody oligosaccharide component. Human IgG1 or IgG3 are N-glycosylated at Asn297 with the majority of the glycans in the biantennary G0, G0F, G1, G1F, G2 or G2F forms. Antibodies produced by non-engineered CHO cells typically have a glycan fucose content of about at least 85%. The removal of the core fucose from the biantennary complex-type oligosaccharides attached to the Fc regions enhances the ADCC of antibodies via

improved FcγRIIIa binding without altering antigen binding or CDC activity. Such modified antibodies can be achieved using different methods reported to lead to the successful expression of relatively high defucosylated antibodies bearing the biantennary complex-type of Fc oligosaccharides such as control of culture osmolality (Konno *et al.*, Cytotechnology 64:(249-65, 2012), application of a variant CHO line Lec13 as the host cell line (Shields *et al.*, J Biol Chem 277:26733-26740, 2002), application of a variant CHO line EB66 as the host cell line (Olivier *et al.*, MAbs ;2(4), 2010; Epub ahead of print; PMID:20562582), application of a rat hybridoma cell line YB2/0 as the host cell line (Shinkawa *et al.*, J Biol Chem 278:3466-3473, 2003), introduction of small interfering RNA specifically against the  $\alpha$  1,6-fucosyltransferase (*FUT8*) gene (Mori *et al.*, Biotechnol Bioeng 88:901-908, 2004), or coexpression of  $\beta$ -1,4-*N*-acetylglucosaminyltransferase III and Golgi  $\alpha$ -mannosidase II or a potent alpha-mannosidase I inhibitor, kifunensine (Ferrara *et al.*, J Biol Chem 281:5032-5036, 2006, Ferrara *et al.*, Biotechnol Bioeng 93:851-861, 2006; Xhou *et al.*, Biotechnol Bioeng 99:652-65, 2008).

In some embodiments, the anti-CD38 antibody has a biantennary glycan structure with fucose content between about 0% to about 15%, for example 15%, 14%, 13%, 12%, 11% 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or 0%.

In some embodiments, the anti-CD38 antibody has a biantennary glycan structure with fucose content of about 50%, 40%, 45%, 40%, 35%, 30%, 25%, 20%, 15%, 14%, 13%, 12%, 11% 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1% or 0%

Substitutions in the Fc and reduced fucose content may enhance the ADCC activity of the anti-CD38 antibody.

“Fucose content” means the amount of the fucose monosaccharide within the sugar chain at Asn297. The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures. These may be characterized and quantified by multiple methods, for example: 1) using MALDI-TOF of N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures) as described in Int Pat. Publ. No. WO2008/077546 2); 2) by enzymatic release of the Asn297 glycans with subsequent derivatization and detection/ quantitation by HPLC (UPLC) with fluorescence detection and/or HPLC-MS (UPLC-MS); 3) intact protein analysis of the native or reduced mAb, with or without treatment of the Asn297 glycans with Endo S or other enzyme that cleaves between the first and the second GlcNAc monosaccharides, leaving the fucose attached to the first GlcNAc; 4) digestion of the antibody to constituent peptides by enzymatic digestion (e.g., trypsin or endopeptidase Lys-C), and subsequent separation, detection and quantitation by HPLC-MS (UPLC-MS); 5) Separation of the

antibody oligosaccharides from the antibody protein by specific enzymatic deglycosylation with PNGase F at Asn 297. The oligosaccharides thus released can be labeled with a fluorophore, separated and identified by various complementary techniques which allow: fine characterization of the glycan structures by matrix-assisted laser desorption ionization (MALDI) mass spectrometry by comparison of the experimental masses with the theoretical masses, determination of the degree of sialylation by ion exchange HPLC (GlycoSep C), separation and quantification of the oligosaccharide forms according to hydrophilicity criteria by normal-phase HPLC (GlycoSep N), and separation and quantification of the oligosaccharides by high performance capillary electrophoresis-laser induced fluorescence (HPCE-LIF).

“Low fucose” or “low fucose content” as used in the application refers to antibodies with fucose content of about 0% to about 15%.

“Normal fucose” or “normal fucose content” as used herein refers to antibodies with fucose content of about over 50%, typically about over 80% or over 85%.

Antibodies that are substantially identical to the antibody comprising the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13 may be used in the methods of the invention. The term “substantially identical” as used herein means that the two antibody heavy chain or light chain amino acid sequences being compared are identical or have “insubstantial differences”. Insubstantial differences are substitutions of 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acids in an antibody heavy chain or light chain that do not adversely affect antibody properties. Percent identity can be determined for example by pairwise alignment using the default settings of the AlignX module of Vector NTI v.9.0.0 (Invitrogen, Carlsbad, CA). The protein sequences of the present invention can be used as a query sequence to perform a search against public or patent databases to, for example, identify related sequences. Exemplary programs used to perform such searches are the XBLAST or BLASTP programs (<http://www.ncbi.nlm.nih.gov>), or the GenomeQuest™ (GenomeQuest, Westborough, MA) suite using the default settings. Exemplary substitutions that can be made to the anti-CD38 antibodies used in the methods of the invention are for example conservative substitutions with an amino acid having similar charge, hydrophobic, or stereochemical characteristics. Conservative substitutions may also be made to improve antibody properties, for example stability or affinity, or to improve antibody effector functions. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acid substitutions may be made, for example, to the heavy or the light chain of the anti-CD38 antibody. Furthermore, any native residue in the heavy or light chain may also be substituted with alanine, as has been

previously described for alanine scanning mutagenesis (MacLennan *et al.*, *Acta Physiol Scand Suppl* 643:55-67, 1998; Sasaki *et al.*, *Adv Biophys* 35:1-24, 1998). Desired amino acid substitutions may be determined by those skilled in the art at the time such substitutions are desired. Amino acid substitutions may be done for example by PCR mutagenesis (U.S. Pat. No. 4,683,195). Libraries of variants may be generated using well-known methods, for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp) and screening the libraries for variants with desired properties. The generated variants may be tested for their binding to CD38 and their ability to induce ADCC using methods described herein.

“Conservative modifications” refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequences. Conservative modifications include amino acid substitutions, additions and deletions. Conservative substitutions are those in which the amino acid is replaced with an amino acid residue having a similar side chain. The families of amino acid residues having similar side chains are well defined and include amino acids with acidic side chains (e.g., aspartic acid, glutamic acid), basic side chains (e.g., lysine, arginine, histidine), nonpolar side chains (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), uncharged polar side chains (e.g., glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine, tryptophan), aromatic side chains (e.g., phenylalanine, tryptophan, histidine, tyrosine), aliphatic side chains (e.g., glycine, alanine, valine, leucine, isoleucine, serine, threonine), amide (e.g., asparagine, glutamine), beta-branched side chains (e.g., threonine, valine, isoleucine) and sulfur-containing side chains (cysteine, methionine). Furthermore, any native residue in the polypeptide may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis (MacLennan *et al.*, (1988) *Acta Physiol Scand Suppl* 643:55-67; Sasaki *et al.*, (1988) *Adv Biophys* 35:1-24). Amino acid substitutions to the antibodies of the invention may be made by known methods for example by PCR mutagenesis (US Patent No. 4,683,195). Alternatively, libraries of variants may be generated for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp). The resulting antibody variants may be tested for their characteristics using assays described herein.

In some embodiments, the anti-CD38 antibody may bind human CD38 with a range of affinities ( $K_D$ ). In one embodiment, the anti-CD38 antibody binds to CD38 with a  $K_D$  equal to or less than about  $1 \times 10^{-8}$  M, for example  $5 \times 10^{-9}$  M,  $1 \times 10^{-9}$  M,  $5 \times 10^{-10}$  M,

$1 \times 10^{-10}$  M,  $5 \times 10^{-11}$  M,  $1 \times 10^{-11}$  M,  $5 \times 10^{-12}$  M,  $1 \times 10^{-12}$  M,  $5 \times 10^{-13}$  M,  $1 \times 10^{-13}$  M,  $5 \times 10^{-14}$  M,  $1 \times 10^{-14}$  M or  $5 \times 10^{-15}$  M, or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art. An exemplary affinity is equal to or less than  $1 \times 10^{-8}$  M. Another exemplary affinity is equal to or less than  $1 \times 10^{-9}$  M.

KinExA instrumentation, ELISA or competitive binding assays known to those skilled in the art. The measured affinity of a particular antibody/ CD38 interaction may vary if measured under different conditions (e.g., osmolarity, pH). Thus, measurements of affinity and other binding parameters (e.g.,  $K_D$ ,  $K_{on}$ ,  $K_{off}$ ) are typically made with standardized conditions and a standardized buffer, such as the buffer described herein. Skilled in the art will appreciate that the internal error for affinity measurements for example using Biacore 3000 or ProteOn (measured as standard deviation, SD) may typically be within 5-33% for measurements within the typical limits of detection. Therefore the term "about" in the context of  $K_D$  reflects the typical standard deviation in the assay. For example, the typical SD for a  $K_D$  of  $1 \times 10^{-9}$  M is up to  $\pm 0.33 \times 10^{-9}$  M.

In some embodiments, the anti-CD38 antibody is a bispecific antibody. The VL and/or the VH regions of existing anti-CD38 antibodies or the VL and VH regions identified *de novo* as described herein may be engineered into bispecific full length antibodies. Such bispecific antibodies may be made by modulating the CH3 interactions in antibody Fc to form bispecific antibodies using technologies such as those described in U.S. Pat. No. 7,695,936; Int. Pat. Publ. No. WO04/111233; U.S. Pat. Publ. No. US2010/0015133; U.S. Pat. Publ. No. US2007/0287170; Int. Pat. Publ. No. WO2008/119353; U.S. Pat. Publ. No. US2009/0182127; U.S. Pat. Publ. No. US2010/0286374; U.S. Pat. Publ. No. US2011/0123532; Int. Pat. Publ. No. WO2011/131746; Int. Pat. Publ. No. WO2011/143545; or U.S. Pat. Publ. No. US2012/0149876.

For example, bispecific antibodies of the invention may be generated *in vitro* in a cell-free environment by introducing asymmetrical mutations in the CH3 regions of two monospecific homodimeric antibodies and forming the bispecific heterodimeric antibody from two parent monospecific homodimeric antibodies in reducing conditions to allow disulfide bond isomerization according to methods described in Intl. Pat. Publ. No. WO2011/131746. In the methods, the first monospecific bivalent antibody (e.g., anti-CD38 antibody) and the second monospecific bivalent antibody are engineered to have certain substitutions at the CH3 domain that promote heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the

hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions may optimally be restored to non-reducing. Exemplary reducing agents that may be used are 2- mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl)phosphine (TCEP), L-cysteine and beta-mercaptoethanol, preferably a reducing agent selected from the group consisting of: 2- mercaptoethylamine, dithiothreitol and tris(2-carboxyethyl)phosphine. For example, incubation for at least 90 min at a temperature of at least 20°C in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

Exemplary CH3 mutations that may be used in a first heavy chain and in a second heavy chain of the bispecific antibody are K409R and/or F405L.

Additional bispecific structures into which the VL and/or the VH regions of the antibodies of the invention may be incorporated are for example Dual Variable Domain Immunoglobulins (DVD) (Int. Pat. Publ. No. WO2009/134776), or structures that include various dimerization domains to connect the two antibody arms with different specificity, such as leucine zipper or collagen dimerization domains (Int. Pat. Publ. No. WO2012/022811, U.S. Pat. No. 5,932,448; U.S. Pat. No. 6,833,441). DVDs are full length antibodies comprising the heavy chain having a structure VH1-linker-VH2-CH and the light chain having the structure VL1-linker-VL2-CL; linker being optional.

In some embodiments, the anti-CD38 antibody is conjugated to a toxin. Conjugation methods and suitable toxins are well known.

In some embodiments, the subject having MM is homozygous for phenylalanine at position 158 of CD16 (FcγRIIIa-158F/F genotype) or heterozygous for valine and phenylalanine at position 158 of CD16 (FcγRIIIa-158F/V genotype). CD16 is also known as the Fc gamma receptor IIIa (FcγRIIIa) or the low affinity immunoglobulin gamma Fc region receptor III-A isoform. Valine/phenylalanine (V/F) polymorphism at FcγRIIIa protein residue position 158 has been shown to affect FcγRIIIa affinity to human IgG. Receptor with FcγRIIIa-158F/F or FcγRIIIa-158F/V polymorphisms demonstrates reduced Fc engagement and therefore reduced ADCC when compared to the FcγRIIIa-158V/V. The lack of or low amount of fucose on human N-linked oligosaccharides improves the ability of the antibodies to induce ADCC due to improved binding of the antibodies to human FcγRIIIa (CD16) (Shields *et al.*, J Biol Chem 277:26733-40, 2002). Patients can be analyzed for their FcγRIIIa polymorphism using routine methods.

In some embodiments, the survivin inhibitor is a small molecule.

In some embodiments, the survivin inhibitor is a polynucleotide.

Survivin inhibitors may inhibit survivin-induced apoptosis by any mechanism, such as inhibiting survivin gene transcription or protein expression, inhibiting survivin protein dimerization, enhancing destabilization or inducing its degradation, etc.

An exemplary survivin small molecule inhibitor is YM155. YM155 binds to the survivin promoter and inhibits its transcription. Other exemplary survivin small molecule inhibitors are, for example, nordihydroguaiaretic acid derivatives as described in U.S. Pat. No. 6,608,108, and molecules described in U.S. Pat. Publ. No. US2012/0122910. Other survivin polynucleotide inhibitors are described, for example, in U.S. Pat. No. 6,838,283, Intl. Pat. Publ. Nos. WO01/057059, WO09/114476 and WO09/044793. Polynucleotide inhibitors include microRNAs (miRNAs), small interference RNAs (siRNAs), allele specific oligos (ASOs) and other polynucleotide inhibitors known in the art.

#### **Administration/ Pharmaceutical Compositions**

In the methods of the invention, the anti-CD38 antibodies may be provided in suitable pharmaceutical compositions comprising the anti-CD38 antibody and a pharmaceutically acceptable carrier. The carrier may be diluent, adjuvant, excipient, or vehicle with which the anti-CD38 antibody is administered. Such vehicles may be liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. For example, 0.4% saline and 0.3% glycine can be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (e.g., filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the molecules or antibodies of the invention in such pharmaceutical formulation may vary widely, i.e., from less than about 0.5%, usually to at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected. Suitable vehicles and formulations, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in e.g. Remington: The Science and Practice of Pharmacy, 21<sup>st</sup> Edition, Troy, D.B. ed., Lipincott Williams and Wilkins, Philadelphia, PA 2006, Part 5, Pharmaceutical Manufacturing pp 691-1092, See especially pp. 958-989.

The mode of administration of the anti-CD38 antibody in the methods of the invention may be any suitable route such as parenteral administration, e.g., intradermal, intramuscular, intraperitoneal, intravenous or subcutaneous, pulmonary, transmucosal (oral, intranasal, intravaginal, rectal) or other means appreciated by the skilled artisan, as well known in the art.

The anti-CD38 antibody in the methods of the invention may be administered to a patient by any suitable route, for example parentally by intravenous (IV) infusion or bolus injection, intramuscularly or subcutaneously or intraperitoneally. IV infusion can be given over for example 15, 30, 60, 90, 120, 180, or 240 minutes, or from 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11 or 12 hours.

The dose given to a patient having CD38-positive hematological malignancy is sufficient to alleviate or at least partially arrest the disease being treated ("therapeutically effective amount") and may be sometimes 0.005 mg to about 100 mg/kg, e.g. about 0.05 mg to about 30 mg/kg or about 5 mg to about 25 mg/kg, or about 4 mg/kg, about 8 mg/kg, about 16 mg/kg or about 24 mg/kg, or for example about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg, but may even higher, for example about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90 or 100 mg/kg.

A fixed unit dose may also be given, for example, 50, 100, 200, 500 or 1000 mg, or the dose may be based on the patient's surface area, e.g., 500, 400, 300, 250, 200, or 100 mg/m<sup>2</sup>. Usually between 1 and 8 doses, (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) may be administered to treat MM, but 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20 or more doses may be given.

The administration of the anti-CD38 antibody in the methods of the invention may be repeated after one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, one month, five weeks, six weeks, seven weeks, two months, three months, four months, five months, six months or longer. Repeated courses of treatment are also possible, as is chronic administration. The repeated administration may be at the same dose or at a different dose. For example, the anti-CD38 antibody in the methods of the invention may be administered at 8 mg/kg or at 16 mg/kg at weekly interval for 8 weeks, followed by administration at 8 mg/kg or at 16 mg/kg every two weeks for an additional 16 weeks, followed by administration at 8 mg/kg or at 16 mg/kg every four weeks by intravenous infusion.

The anti-CD38 antibodies may be administered in the methods of the invention by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

For example, anti-CD38 antibodies in the methods of the invention may be provided as a daily dosage in an amount of about 0.1-100 mg/kg, such as 0.5, 0.9, 1.0, 1.1,

1.5, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 40, 45, 50, 60, 70, 80, 90 or 100 mg/kg. per day, on at least one of day 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, or 40, or alternatively, at least one of week 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19 or 20 after initiation of treatment, or any combination thereof, using single or divided doses of every 24, 12, 8, 6, 4, or 2 hours, or any combination thereof.

Anti-CD38 antibodies in the methods of the invention may also be administered prophylactically in order to reduce the risk of developing cancer, delay the onset of the occurrence of an event in cancer progression, and/or reduce the risk of recurrence when a cancer is in remission. This may be especially useful in patients wherein it is difficult to locate a tumor that is known to be present due to other biological factors.

The anti-CD38 antibody in the methods of the invention may be lyophilized for storage and reconstituted in a suitable carrier prior to use. This technique has been shown to be effective with conventional protein preparations and well known lyophilization and reconstitution techniques can be employed.

In the methods of the invention, the anti-CD38 antibody is administered in combination with a survivin inhibitor.

In the methods of the invention, the anti-CD38 antibody is administered in combination with a survivin inhibitor YM155.

YM155 used in the methods of the invention is readily available according to the processes of production as disclosed in International Publication Intl. Pat. Publ. Nos. WO01/60803 and WO2004/092160.

YM155 may be administered orally or parenterally, or intravenously. In this connection, the injection preparation for intravenous administration includes those containing sterile aqueous or non-aqueous solutions, suspensions, and emulsions. The aqueous solvent includes, for example, distilled water for injection and physiological saline. The non-aqueous solvent includes, for example, propylene glycol, polyethylene glycol, vegetable oils such as olive oil, alcohols such as ethanol, polysorbate 80, and the like. Such compositions may contain further tonicity adjusting agents, antiseptics, moistening agents, emulsifying agents, dispersing agents, stabilizers, and solubilizing agents. These may be sterilized, for example, by filtration through a bacterial filter, blending of sterilizers or irradiation. Alternatively, it is possible to prepare a germ-free solid composition and dissolve or suspend it in sterile water or a sterile solvent for injection immediately before use.

In intravenous administration, YM155 may be administered, for example, at 0.1-20 mg/m<sup>2</sup>/day, such as at 1-10 mg/m<sup>2</sup>/day, once a day or divided in plural doses, or continuously by infusion (continuous instillation). YM155 may be infused at 3-10 mg/m<sup>2</sup>/day continuously for a period of 4 days to 20 days, for example 4 days to 14 days, or 5 days, 7 days, 10 days or 14 days, and or for 7 days. When the administration is further continued, a medication cycle may be employed comprising a term of drug holidays of 1 day to 2 months, 7 days to 21 days, or 14 days, after termination of the above term of medication. Alternatively, YM155 may be administered continuously by infusion at a dose of 3-8 mg/m<sup>2</sup>/day for 7 days, followed by drug holidays of 14 days; this cycle as one cycle is repeated depending on the conditions. The frequency of administration, dosage, time of infusion, medication cycle, and the like, may be determined properly according to individual cases considering the kind of anticancer agent, state of the patients, age, gender, etc.

In the methods of the invention, the combination of the anti-CD38 antibody and survivin inhibitor may be administered over any convenient timeframe. For example, the anti-CD38 antibody and survivin inhibitor may be administered to a patient on the same day. However, the anti-CD38 antibody and survivin may also be administered on alternating days or alternating weeks or months, and so on. In some methods, the anti-CD38 antibody and survivin inhibitor may be administered with sufficient proximity in time that they are simultaneously present (e.g., in the serum) at detectable levels in the patient being treated. In some methods, an entire course of treatment with the anti-CD38 antibody consisting of a number of doses over a time period is followed or preceded by a course of treatment with survivin inhibitor, consisting of a number of doses. A recovery period of 1, 2 or several days or weeks may be used between administration of the anti-CD38 antibody and survivin inhibitor.

Anti-CD38 antibody in combination with survivin inhibitor may be administered together with any form of radiation therapy including external beam radiation, intensity modulated radiation therapy (IMRT) and any form of radiosurgery including Gamma Knife, Cyberknife, Linac, and interstitial radiation (e.g., implanted radioactive seeds, GliSite balloon), and/or with surgery.

**Subcutaneous administration of pharmaceutical compositions comprising an antibody that specifically binds CD38 and a hyaluronidase**

The anti-CD38 antibody may be administered as a pharmaceutical composition comprising the anti-CD38 antibody and a hyaluronidase subcutaneously.

The concentration of the anti-CD38 antibody in the pharmaceutical composition administered subcutaneously may be about 20 mg/ml.

The pharmaceutical composition administered subcutaneously may comprise between about 1,200 mg – 1,800 mg of the anti-CD38 antibody.

The pharmaceutical composition administered subcutaneously may comprise about 1,200 mg of the anti-CD38 antibody.

The pharmaceutical composition administered subcutaneously may comprise about 1,600 mg of the anti-CD38 antibody.

The pharmaceutical composition administered subcutaneously may comprise about 1,800 mg of the anti-CD38 antibody.

The pharmaceutical composition administered subcutaneously may comprise between about 30,000 U – 45,000 U of the hyaluronidase.

The pharmaceutical composition administered subcutaneously may comprise about 1,200 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

The pharmaceutical composition administered subcutaneously may comprise about 1,800 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

The pharmaceutical composition administered subcutaneously may comprise about 1,600 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

The pharmaceutical composition administered subcutaneously may comprise about 1,600 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

The pharmaceutical composition administered subcutaneously may comprise the hyaluronidase rHuPH20 having the amino acid sequence of SEQ ID NO: 23.

rHuPH20 is a recombinant hyaluronidase (HYLENEX® recombinant) and is described in Int. Pat. Publ. No. WO2004/078140.

Hyaluronidase is an enzyme that degrades hyaluronic acid (EC 3.2.1.35) and lowers the viscosity of hyaluronan in the extracellular matrix, thereby increasing tissue permeability.

**SEQ ID NO: 23**

MGVLKFKHIFRSFVKSSGVSQIVFTLLIPCCCLTLNFRAPPVIPNVPFLWAWNAPS  
EFCLGKFDEPLDMSLFSFIGSPRINATGQGVTFYVDRLGYYPYIDSITGVTVNGGIP

QKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRS  
 IELVQQQNVQLSLTEATEKAKQEFKAGKDFLVETIKLGKLLRPNHLWGYLFPD  
 CYNHHYKKPGYNGSCFNVEIKRNDLWSLWNESTALYPSIYLNTQQSPVAATLY  
 VRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGA  
 SGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAMCSQVLCQEQGVCI  
 KNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFCSCYSTLSCK  
 EKADVKTDAVDVCIADGVCIDAFKPPMETEEPQIFYNASPSTLSATMFIVSILFLI  
 ISSVASL

The administration of the pharmaceutical composition comprising the anti-CD38 antibody and the hyaluronidase may be repeated after one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, four weeks, five weeks, six weeks, seven weeks, two months, three months, four months, five months, six months or longer. Repeated courses of treatment are also possible, as is chronic administration. The repeated administration may be at the same dose or at a different dose. For example, the pharmaceutical composition comprising the anti-CD38 antibody and the hyaluronidase may be administered once weekly for eight weeks, followed by once in two weeks for 16 weeks, followed by once in four weeks. The pharmaceutical compositions to be administered may comprise about 1,200 mg of the anti-CD38 antibody and about 30,000 U of hyaluronidase, wherein the concentration of the antibody that specifically binds CD38 in the pharmaceutical composition is about 20 mg/ml. The pharmaceutical compositions to be administered may comprise about 1,800 mg of the anti-CD38 antibody and about 45,000 U of hyaluronidase. The pharmaceutical compositions to be administered may comprise about 1,600 mg of the anti-CD38 antibody and about 30,000 U of hyaluronidase. The pharmaceutical compositions to be administered may comprise about 1,600 mg of the anti-CD38 antibody and about 45,000 U of hyaluronidase.

The pharmaceutical composition comprising the anti-CD38 antibody and the hyaluronidase may be administered subcutaneously to the abdominal region.

The pharmaceutical composition comprising the anti-CD38 antibody and the hyaluronidase may be administered in a total volume of about 80 ml, 90 ml, 100 ml, 110 ml or 120 ml.

For administration, 20 mg/ml of the anti-CD38 antibody in 25 mM sodium acetate, 60 mM sodium chloride, 140 mM D-mannitol, 0.04% polysorbate 20, pH 5.5 may be mixed with rHuPH20, 1.0 mg/mL (75-150 kU/mL) in 10 mM L-Histidine, 130 mM

NaCl, 10 mM L-Methionine, 0.02% Polysorbate 80, pH 6.5 prior to administration of the mixture to a subject.

#### **Further embodiments of the invention**

1. An anti-CD38 antibody for use in treating a subject having a CD38-positive hematological malignancy, in combination with a survivin inhibitor.
2. A survivin inhibitor for use in treating a subject having a CD38-positive hematological malignancy, in combination with an anti-CD38 antibody.
3. The combination of an anti-CD38 antibody and a survivin inhibitor for use in treating a subject having a CD38-positive hematological malignancy.
4. The anti-CD38 antibody for use according to embodiment 1, the survivin inhibitor for use according to embodiment 2, or the combination according to embodiment 3, wherein CD38-positive hematological malignancy is multiple myeloma (MM), acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL).
5. The anti-CD38 antibody for use according to embodiment 1 or 4, the survivin inhibitor for use according to embodiment 2 or 4, or the combination according to embodiment 3 or 4, wherein CD38-positive hematological malignancy is a plasma cell disease.
6. The anti-CD38 antibody for use according to embodiment 1, 4 or 5, the survivin inhibitor for use according to embodiment 2, 4 or 5, or the combination according to embodiment 3, 4 or 5, wherein the plasma cell disease is light chain amyloidosis (AL), multiple myeloma (MM) or Waldenstrom's macroglobulinemia.
7. The anti-CD38 antibody for use according to any one of embodiments 1 or 4-6, the survivin inhibitor for use according to any one of embodiments 2 or 4-6, or the combination according to any one of embodiments 3 or 4-6, wherein the plasma cell disease is MM.
8. The anti-CD38 antibody for use according to any one of embodiments 1 or 4-7, the survivin inhibitor for use according to any one of embodiments 2 or 4-7, or the combination according to any one of embodiments 3 or 4-7, wherein the anti-

CD38 antibody induces CD38-positive cell killing by antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC) or apoptosis.

9. The anti-CD38 antibody for use according to any one of embodiments 1 or 4-8, the survivin inhibitor for use according to any one of embodiments 2 or 4-8, or the combination according to any one of embodiments 3 or 4-8, wherein the anti-CD38 antibody is
  - a. of IgG1, IgG2, IgG3 or IgG4 isotype; or
  - b. is of IgG1 isotype.
10. The anti-CD38 antibody for use according to any one of embodiments 1 or 4-9, the survivin inhibitor for use according to any one of embodiments 2 or 4-9, or the combination according to any one of embodiments 3 or 4-9, wherein the anti-CD38 antibody
  - a. competes for binding to CD38 with an antibody comprising a heavy chain variable region (VH) of SEQ ID NO: 4 and a light chain variable region (VL) of SEQ ID NO: 5;
  - b. binds to the region SKRNIQFSCCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1);
  - c. comprises the heavy chain complementarity determining regions (HCDR) 1 (HCDR1), 2 (HCDR2) and 3 (HCDR3) sequences of SEQ ID NOs: 6, 7 and 8, respectively;
  - d. comprises the light chain complementarity determining regions (LCDR) 1 (LCDR1), 2 (LCDR2) and 3 (LCDR3) sequences of SEQ ID NOs: 9, 10 and 11, respectively;
  - e. comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 sequences of SEQ ID NOs: 6, 7, 8, 9, 10 and 11, respectively;
  - f. comprises a heavy chain variable region (VH) comprising an amino acid sequence that is 95%, 96%, 97%, 98%, 99% or 100% identical to that of SEQ ID NO: 4 and a light chain variable region (VL) comprising an amino acid sequence that is 95%, 96%, 97%, 98%, 99% or 100% identical to that of SEQ ID NO: 5;
  - g. comprises the heavy chain variable region (VH) of SEQ ID NO: 4 and the light chain variable region (VL) of SEQ ID NO: 5;

- h. comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 of:
    - i. the VH of SEQ ID NO: 14 and the VL of SEQ ID NO: 15;
    - ii. the VH of SEQ ID NO: 16 and the VL of SEQ ID NO: 17;
    - iii. the VH of SEQ ID NO: 18 and the VL of SEQ ID NO: 19; or
    - iv. the VH of SEQ ID NO: 20 and the VL of SEQ ID NO: 21; or
  - i. comprises:
    - i. the VH of SEQ ID NO: 14 and the VL of SEQ ID NO: 15;
    - ii. the VH of SEQ ID NO: 16 and the VL of SEQ ID NO: 17;
    - iii. the VH of SEQ ID NO: 18 and the VL of SEQ ID NO: 19; or
    - iv. the VH of SEQ ID NO: 20 and the VL of SEQ ID NO: 21.
- 11.** The anti-CD38 antibody for use according to any one of embodiments 1 or 4-10, the survivin inhibitor for use according to any one of embodiments 2 or 4-10, or the combination according to any one of embodiments 3 or 4-10, wherein the anti-CD38 antibody comprises the heavy chain variable region (VH) of SEQ ID NO: 4 and the light chain variable region (VL) of SEQ ID NO: 5.
- 12.** The anti-CD38 antibody for use according to any one of embodiments 1 or 4-11, the survivin inhibitor for use according to any one of embodiments 2 or 4-11, or the combination according to any one of embodiments 3 or 4-11, wherein the survivin inhibitor is a small molecule, or a polynucleotide or a vaccine.
- 13.** The anti-CD38 antibody for use according to any one of embodiments 1 or 4-12, the survivin inhibitor for use according to any one of embodiments 2 or 4-12, or the combination according to any one of embodiments 3 or 4-12, wherein the survivin inhibitor is YM155.
- 14.** The anti-CD38 antibody for use according to any one of embodiments 1 or 4-13, the survivin inhibitor for use according to any one of embodiments 2 or 4-13, or the combination according to any one of embodiments 3 or 4-13, wherein the anti-CD38 antibody and the survivin inhibitor are administered simultaneously, sequentially or separately.
- 15.** An anti-CD38 antibody for use in treating a subject having a CD38-positive hematological malignancy, in combination with a survivin inhibitor, wherein the anti-CD38 antibody comprises the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2 and the LCDR3 sequences of SEQ ID NOs: 6, 7, 8, 9, 10 and 11, respectively;

16. The anti-CD38 antibody for use according to embodiment 15, wherein the anti-CD38 antibody comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5.
17. The anti-CD38 antibody for use according to embodiment 15 or 16, wherein the anti-CD38 antibody is of IgG1 isotype.
18. The anti-CD38 antibody for use according to any one of embodiments 15-17, wherein the anti-CD38 antibody comprises a heavy chain of SEQ ID NO: 12 and a light chain of SEQ ID NO: 13.
19. The anti-CD38 antibody for use according to any one of embodiments 15-18, wherein the anti-CD38 antibody and the survivin inhibitor are administered simultaneously, sequentially or separately.
20. The anti-CD38 antibody for use according to any one of embodiments 15-19, wherein the anti-CD38 antibody is administered intravenously.
21. The anti-CD38 antibody for use according to any one of embodiments 15-19, wherein the anti-CD38 antibody is administered subcutaneously in a pharmaceutical composition comprising the anti-CD38 antibody and a hyaluronidase.
22. The anti-CD38 antibody for use according to embodiment 21, wherein the hyaluronidase is rHuPH20 of SEQ ID NO: 23.
23. The anti-CD38 antibody for use according to any one of embodiments 15-22, wherein the CD38-positive hematological malignancy is multiple myeloma.

While having described the invention in general terms, the embodiments of the invention will be further disclosed in the following examples that should not be construed as limiting the scope of the claims.

## **Materials and Methods**

### **Cells and Cell culture**

#### **Bone marrow mononuclear cells (BM-MNCs) and peripheral blood mononuclear cells (PBMCs)**

BM aspirates from MM patients or healthy individuals, as well as PB from healthy individuals were collected using protocols and procedures approved by the institutional medical ethical committee in accordance with the declaration of Helsinki. Healthy Donor (HD)-PBMCs and BM-MNCs were isolated by Ficoll-Hypaque density-gradient

centrifugation from PB samples or BM aspirates respectively. PBMCs were used directly as effector cells in ADCC experiments; BM-MNCs were cryopreserved until use.

#### Multiple Myeloma (MM) cell lines

The luciferase (Luc)-transduced human MM cell lines RPMI-8226 and UM9 were maintained in RPMI1640 (Invitrogen), supplemented with 10% fetal bovine serum (FBS; Integro BV) and antibiotics (penicillin/streptomycin; Life Technologies) at 37°C in a humidified atmosphere containing 5% CO<sub>2</sub>.

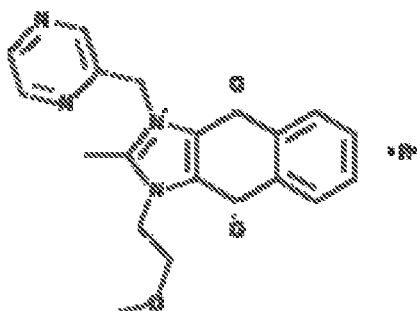
#### Bone marrow stromal cells (BMSC)

Adherent stromal cells were isolated and cultured from BM-MNCs of healthy individuals (hBMSC) or of MM patients (pBMSC) by plastic adherence. Cells were cultured in Optimem (Invitrogen) with 5% platelet lysate, heparin and antibiotics. hBMSCs were used in experiments until passage six and pBMSC were used after passage one or two.

#### Reagents

YM155 (Sepantronium Bromide; 4,9-Dihydro-1-(2-methoxyethyl)-2-methyl-4,9-dioxo-3-(2-pyrazinylmethyl)-1H-naphth[2,3-d]imidazolium bromide; CAS 781661-94-7) (Selleck Chemicals) was dissolved in dimethyl sulfoxide (DMSO) at a concentration of 1mM and aliquoted for storage until use. YM155 was diluted in culture medium to the concentrations indicated in each experiment.

YM155; Formula I:



I

**Compartment specific bioluminescence based Antibody dependent cell-mediated cytotoxicity (ADCC) against Multiple Myeloma cell lines (“compartment-specific BLI-based cytotoxicity assays”)**

hBMSC were plated at a density of  $1 \times 10^4$  cells/well in white opaque flat-bottomed 96-well plates (Costar) in 100  $\mu$ l culture medium. After an adherence period of six hours, luc-transduced MM cell lines were added at a density of  $1 \times 10^4$  cells/well in BMSC-coated or uncoated wells. In experiments in which YM155 was tested, YM155 was added at indicated concentrations together with MM cells. After 16-20 hours, daratumumab was added at indicated concentrations and left for 15 minutes at room temperature. Freshly isolated PBMC from healthy individuals were then added as effector cells at the indicated effector target ratios. 4 hours after addition of PBMC, 125  $\mu$ g/ml beetle luciferin (Promega) was added and the bioluminescent signal emitted from surviving MM cells was determined within 20 minutes using a luminometer (SpectraMax, Molecular Devices). The percentage survival of MM cells was calculated using the formula: % survival = (mean bioluminescent signal in the absence of PBMCs / mean bioluminescent signal in the presence of PBMCs)  $\times 100\%$ . In these assays the survival of MM cells is a direct reflection of ADCC mediated lysis and correlates with classical chromium release assays as described in McMillin *et al.*, Nat Med 16:483-489, 2010.

**FACS based ADCC assay in Multiple Myeloma BM-MNC**

Frozen BM-MNCs from MM patients with 15-35% CD138<sup>+</sup> MM cells were used in FACS-based ADCC assays. The cells were thawed and cultured in 10% HS in RPMI. After 16-20 h, BM-MNCs were counted by trypan blue exclusion and  $4 \times 10^4$  cells/well were plated in 96 round bottom plates. Daratumumab and/or YM155 were added in the wells as indicated per experiment. After 24 hours, cells were stained with fluorescent conjugated anti-CD138, anti-CD38, anti-CD56 and anti-CD3 antibodies and the survival of primary CD138<sup>+</sup> MM cells in the BM-MNCs was determined by FACS as previously described (Groen *et al.*, Blood 120:e9-e16, 2012). Percentage lysis of MM cells was deduced using the following formula: % lysis cells = 1 - (counts of surviving CD138<sup>+</sup> cells in treated wells/counts of number of surviving CD138<sup>+</sup> cells in control wells)  $\times 100\%$ .

### Flow cytometry

To determine the level of CD38 expression on MM cells, MM cells were cultured alone or with BMSCs and incubated with CD38 fluorescein conjugated antibody. The cells were additionally stained with CD105 as a marker for BMSCs. The CD38 expression on CD105 negative cells was determined by FACS as described (de Haart et al., Clin Cancer Res 19:5591-601, 2013).

### *In vivo* tumor targeting experiments

Hybrid scaffolds consisting of three 2- to 3-mm biphasic calcium phosphate particles coated with HD-BMSC were *in vitro* loaded with Luc<sup>+</sup> MM cell line UM9 (1x10<sup>6</sup> cells /scaffold) before s.c. implantation into RAG2<sup>-/-</sup>γc<sup>-/-</sup> mice as described previously (Groen et al., Blood 120:e9-e16, 2012). Ten days after implantation, mice with tumors growing in the scaffolds were treated with vehicle control, daratumumab + PBS or daratumumab+ YM155. Each mice, including the control group, received in addition T cell-depleted HD-PBMCs (5x10<sup>6</sup> cells) as a source of human NK cells to induce ADCC. PBS and YM155-diluted in PBS were administered using subcutaneous infusion pumps (Alzet 1007D) delivering 1 mg/kg/d of the drug continuously. Pumps were removed after 10 days. BLI was performed as described previously (Spaapen et al., Clin Cancer Res 16:5481-88, 2010; Rozemuller et al., Haematologica 93:1049-57, 2008).

### Granzyme B Enzyme linked immunosorbent assay (ELISA)

The granzyme B (GzB) content of cell-free supernatants was determined using a commercial ELISA kit (Pelipair, Sanquin, Amsterdam, NL) according to the manufacturer's instructions.

### Example 1. Bone Marrow stromal cells confer protection against antibody-dependent cellular cytotoxicity of multiple myeloma cells

Since the stromal cells of bone marrow (BM) microenvironment protect MM cells against CTL and NK mediated cytotoxicity, it was evaluated whether a similar protective effect occurs against antibody-dependent cellular cytotoxicity (ADCC) induced by daratumumab.

Induction of ADCC by healthy donor BMSCs against two CD38<sup>+</sup> luciferase transduced MM cell lines, UM9 and RPMI was tested with serial concentrations of daratumumab in the presence or absence of HD-PBMCs as effector cells in compartment-specific BLI-based cytotoxicity assays. In the absence of BMSCs, daratumumab mediated ADCC in both MM cell lines in a dose dependent fashion. Both cell lines were less sensitive to daratumumab-induced ADCC in the presence of BMSCs. **Figure 1A** shows the effect of BMSCs on daratumumab-induced ADCC in UM9 cells, and **Figure 1B** shows the effect of BMSCs on daratumumab-induced ADCC in RPMI-8226 cells.

Ability of BMSCs to protect primary MM cells from daratumumab-induced ADCC was also evaluated in a FACS-based ADCC assay using methodology described above. In the assays, BM-MNCs containing at least 15% CD138<sup>+</sup> malignant plasma cells and sufficient numbers of autologous effector NK cells were incubated with daratumumab to induce ADCC. The BM-MNCs were tested either alone or in co-culture with autologous BMSCs to evaluate the effect of BMSCs. FACS-based viability assay was performed after 24 hours of culture to determine CD138<sup>+</sup> surviving cells and calculate lysis. Induction of ADCC of primary MM cells was less efficient in the presence of autologous MM-BMSCs in both donors tested (**Figure 2A** and **Figure 2B**), indicating that the stromal cells of the tumor microenvironment induced a resistance to daratumumab therapy.

**Example 2. BMSC-induced suppression of ADCC is not caused by CD38 down-regulation or NK cell suppression.**

Possible changes in CD38 surface expression and NK cell activation were evaluated to understand the mechanisms of BMSC-mediated protection against ADCC.

MM cell lines UM9 and RPMI-8226 were cultured in the presence or absence of healthy donor BMSCs. Co-culture of MM cells with BMSCs did not down-regulate the CD38 expression levels on either MM cell line (data not shown).

Since BMSCs are known to produce several immunosuppressive factors such as IDO, TGF- $\beta$  or PGE-2, the protection against ADCC by BMSCs could be due to the suppression of NK-cell activation, which would reduce their ability to degranulate and release granzyme B and perforin in the immune synapses to kill their targets. To that end, effect of BMSCs on NK cell activation by daratumumab was determined using daratumumab-mediated granzyme B excretion as a marker for NK cell activity. Granzyme B levels were in general higher in the supernatants in the presence of BMSCs (data not

shown). Hence, BMSC-mediated protection against ADCC was likely not due to NK cell suppression.

**Example 3. Survivin inhibition abrogates BMSC-mediated protection against ADCC and provides synergistic ADCC-mediated killing of multiple myeloma cells with daratumumab**

BMSCs have been shown to protect MM cells against CTL lysis by up-regulation of survivin in MM cells. Modulation of survivin was evaluated as a possible mechanism for BMSC-mediated protection against ADCC induced by daratumumab using YM155, a small molecule inhibitor of survivin.

Effect of YM155 on NK cell viability was evaluated first. BM-MNCs of MM patients were cultured in the presence of different doses of YM155 for 24 hours. Viability of MM cells (CD138<sup>+</sup> cells) and NK cells (CD3<sup>+</sup>CD138<sup>-</sup>CD56<sup>+</sup> cells) were determined by FACS. NK cells were not affected at YM155 doses which already showed some toxicity to MM cells (Figure 3).

Effect of daratumumab, YM155, or a combination of daratumumab and YM155 was evaluated in RPMI-8226 cells and in two MM patient samples using YM155 concentrations shown to be non-toxic to NK cells.

In the assays, daratumumab and YM155 were used at a concentration of 0.3 µg/ml and 1 nM, respectively, for RPMI-8226 cells and at a concentration of 1 µg/ml and 120 nM for MM patient samples, respectively. Healthy donor PBMCs were used as effector cells at effector:target cell ratio of 40:1 for RPMI-8226 cells and 30:1 for MM patient samples.

In RPMI-8226 cells, daratumumab alone or YM155 alone induced lysis of about 20% of cells in the absence of BMSCs. In the presence of BMSCs, daratumumab induced lysis of about 10% of cells, whereas YM155 had no effect. Combination of daratumumab and YM155 provided a synergistic effect inducing lysis of about 50% of cells in the absence of BMSCs, and inducing lysis of about 45% of cells in the presence of BMSCs (Figure 4A). The synergistic effect of the combination of daratumumab and YM155 in the BMSCs was about 5-fold. Similarly, the combination of daratumumab and YM155 provided a synergistic effect in the MM patient sample 1 using 120 nM YM155 (Figure 4B), a moderate synergistic effect in the MM patient sample 2 using a lower amount of YM155 (64 nM) (Figure 4C), and in a combined sample of MM cells derived from 4 patients (Figure 4D). YM155 therefore abrogated the protective effects of BMSCs on daratumumab-mediated ADCC in MM cells and cell lines.

Thus, survivin up-regulation may be an important mechanism of suppression of ADCC-mediated killing of MM cells, which can be prevented by pharmacological modulation of survivin.

**Example 4. *In vivo* antitumor effect of daratumumab and YM155 combination therapy**

The *in vivo* relevance of the combination of daratumumab with YM155 was tested in the preclinical xenograft model in RAG2<sup>-/-</sup>γc<sup>-/-</sup> mice, in which MM tumors were grown in a humanized BM-like niche created by subcutaneous implantation of ceramic scaffolds coated with human BMSCs. Hybrid scaffolds coated with human MSCs and loaded with luciferase transduced MM cell line UM9 were implanted subcutaneously on the back of RAG2<sup>-/-</sup>γc<sup>-/-</sup> mice (4 scaffolds per mice). Ten days after implantation, the growing tumors were visualized and quantified by BLI. Different groups of mice (n=4) were then either treated with vehicle control (control) or treated with daratumumab, YM155 or daratumumab plus YM155. YM155 or its vehicle, PBS was delivered with subcutaneous infusion pumps at a rate of 1 mg/kg/d YM155 for 10 days. Each mouse, including the control group, received T cell-depleted HD-PBMCs (5x10<sup>6</sup> cells) as a source of human NK cells to induce ADCC. Mice were monitored weekly by BLI. **Figure 5** shows the relative tumor growth for each group. Statistical differences between mice treated with daratumumab and mice treated with daratumumab plus YM155 were calculated using the Mann-Whitney U-test. Daratumumab had a marginal effect on tumor growth. The anti-MM effect was more pronounced with YM155, which furthermore showed strong synergism with daratumumab to achieve significantly improved anti-MM effects. These results suggested that a clinical benefit may be expected from the combination of daratumumab with the survivin inhibitor YM155.

The results presented demonstrate that suppressing survivin levels with a small molecule YM155 not only improved daratumumab-mediated ADCC in the absence of BMSCs, but importantly abrogated the ADCC resistance induced by BMSCs. The addition of YM155 to daratumumab demonstrated enhanced antitumor effects also in the absence of BMSCs, which suggest the potential benefits of YM155-daratumumab combination therapy even if the MM cells are not in direct contact with BMSCs, such as in plasma cell leukemia. Moreover, the significant improvement of ADCC, up to a four-fold improvement of MM cell lysis, in the presence of BMSCs, suggests that a larger benefit from combining daratumumab therapy with YM155 can be achieved for MM cells which

reside in the BM. It was also demonstrated that YM155 treatment does not negatively interfere with NK cell functions or viability, which is a prerequisite to consider clinical application of this combination therapy.

The efficacy of daratumumab in combination with YM155 was demonstrated in multiple myeloma, however, it can be extrapolated that the combination therapy may also be beneficial for other hematological tumors that express CD38, especially for those that mainly reside in the BM. In this respect AML is an eminent candidate, since AML cells not only express high levels of CD38, but also high levels of survivin, which is a predictor for poor clinical outcome. Another potential candidate for combination therapy might be CLL, since CLL cells have high survivin expression in the BM and CD38 is expressed in some patients. High CD38 and survivin expression in about 50% of non-Hodgkin lymphomas, makes this disease also relevant for evaluation of the efficacy of YM155-daratumumab combination. Altogether, a combination of daratumumab and YM155 may be broadly applicable for a wide range of hematological tumors.

## CLAIMS

What is claimed is:

1. Use of an anti-CD38 antibody and a survivin inhibitor in the manufacture of a medicament for the treatment of a subject having a CD38-positive hematological malignancy, wherein the anti-CD38 antibody is of IgG1 isotype and comprises a heavy chain complementarity determining region (HCDR) 1, a HCDR2 and a HCDR3 of SEQ ID NOs: 6, 7 and 8, respectively, and a light chain complementarity determining region (LCDR) 1, a LCDR2 and a LCDR3 of SEQ ID NOs: 9, 10 and 11, respectively, and wherein the survivin inhibitor is YM155.
2. The use of claim 1, wherein the CD38-positive hematological malignancy is multiple myeloma (MM), acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma (NHL), diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), follicular lymphoma (FL), mantle-cell lymphoma (MCL), acute myeloid leukemia (AML) or chronic lymphocytic leukemia (CLL).
3. The use of claim 1, wherein the CD38-positive hematological malignancy is a plasma cell disease.
4. The use of claim 3, wherein the plasma cell disease is light chain amyloidosis (AL), multiple myeloma (MM) or Waldenstrom's macroglobulinemia.
5. The use of claim 4, wherein the plasma cell disease is MM.
6. The use of any one of claims 1-5, wherein the anti-CD38 antibody comprises a heavy chain variable region (VH) comprising an amino acid sequence that is 95%, 96%, 97%, 98%, 99% or 100% identical to that of SEQ ID NO: 4 and a light chain variable region (VL) comprising an amino acid sequence that is 95%, 96%, 97%, 98%, 99% or 100% identical to that of SEQ ID NO: 5.
7. The use of claim 6, wherein the anti-CD38 antibody comprises the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5.

8. The use of any one of claims 1-7, wherein the anti-CD38 antibody induces CD38-positive cell killing by antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement-dependent cytotoxicity (CDC) or apoptosis.
9. The use of claim 1, wherein the anti-CD38 antibody and the survivin inhibitor in the form of the medicament are to be administered simultaneously.
10. The use of claim 1, wherein the anti-CD38 antibody and the survivin inhibitor in the form of the medicament are to be administered sequentially or separately.
11. The use of claim 9 or claim 10, wherein the anti-CD38 antibody in the form of the medicament is to be administered intravenously.
12. The use of claim 9 or claim 10, wherein the anti-CD38 antibody in the form of the medicament is to be administered subcutaneously in a pharmaceutical composition comprising the anti-CD38 antibody and a hyaluronidase.