

COMBINATION THERAPIES WITH ANTI-CD38 ANTIBODIES

FIELD OF THE INVENTION

The present invention relates to combination therapies with anti-CD38 antibodies and all-trans retinoic acid.

BACKGROUND OF THE INVENTION

B-cell malignancies include B-cell chronic lymphocytic leukemia, mantle cell lymphoma, Burkitt lymphoma, follicular lymphoma, diffuse large B-cell lymphoma, multiple myeloma, Hodgkin's lymphoma, hairy cell leukemia, primary effusion lymphoma and AIDS-related Non-Hodgkin's Lymphoma. B-cell malignancies comprise more than 85% of diagnosed lymphomas.

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.

CD38 is a type II membrane protein having function in receptor-mediated adhesion and signaling as well as mediating calcium mobilization via its ecto-enzymatic activity, catalyzing formation of cyclic ADP-ribose (cADPR) from NAD^+ and also hydrolyzing cADPR into ADP-ribose (ADPR). CD38 mediates cytokine secretion and activation and proliferation of lymphocytes (Funaro *et al.*, J Immunology 145:2390-6, 1990; Terhorst *et al.*, Cell 77:1-80, 1981; Guse *et al.*, Nature 398:70-3, 1999), and via its NAD glycohydrolase activity regulates extracellular NAD^+ levels which have been implicated in modulating the regulatory T-cell compartment (Adriouch *et al.*, 14:1284-92, 2012; Chiarugi *et al.*, Nature Reviews 12:741-52, 2012).

CD38 is expressed on MM malignant plasma cells, and is implicated in various hematological malignancies.

Currently available therapies for MM include chemotherapy, stem cell transplantation, Thalomid® (thalidomide), Revlimid® (lenalidomide), Velcade® (bortezomib), 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%. 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, yet 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- α) alone or in combination with steroids. Thus, there is a need for additional therapies for the treatment of multiple myeloma and other B-cell malignancies.

SUMMARY OF THE INVENTION

One embodiment of the invention is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to a patient in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC).

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows that all-trans retinoic acid (ATRA) enhances CD38 expression on multiple myeloma (MM) cell lines in a dose dependent manner. MM cell lines RPMI8226, UM9 and XG1 were incubated with RPMI-1640 medium alone or with 0-25 nM ATRA for 48 hours and then harvested to determine CD38 expression by flow cytometry. The graph shows results of one representative experiment. The Y axis shows the fold increase of mean fluorescent intensity (MFI) of CD38 surface expression.

Figure 1B shows that ATRA enhances CD38 expression on MM cell lines in a time dependent manner. MM cell lines RPMI8226, UM9 and XG1 were incubated with RPMI-1640 medium alone or with 10 nM ATRA for 24, 48, 72 or 96 hours and then harvested to determine CD38 expression by flow cytometry. The graph shows results of one representative experiment. The Y axis shows the fold increase of mean fluorescent intensity (MFI) of CD38 surface expression.

Figure 2 shows that ATRA enhances CD38 expression on bone marrow mononuclear cells (BM-MNCs) from MM patients *ex vivo*. BM-MNCs from 26 MM patients were incubated with RPMI-1640 medium alone or with 10 nM ATRA for 48 hours and then

harvested to determine CD38 expression by flow cytometry. The Y axis shows the MFI of CD38 surface expression. Medium: medium at 0 hours. ns: not significant. *** $p < 0.001$; **** $p < 0.0001$.

Figure 3A shows daratumumab-induced complement-dependent cytotoxicity (CDC) (top panel) and antibody-dependent cell mediated cytotoxicity (ADCC) (bottom panel) in MM XG1 cell line pretreated with or without 10 nM ATRA for 48 hours prior to performing CDC or ADCC in the presence of 10 $\mu\text{g/ml}$ daratumumab. The Y axis shows the percent (%) CDC or ADCC. Data show the mean and SEM of at least three experiments. p-values between the indicated groups were calculated using a paired student's *t* test. Dara: daratumumab; * $p < 0.05$; ** $p < 0.01$.

Figure 3B shows daratumumab-induced CDC (top panel) and ADCC (bottom panel) in MM RPMI8226 cell line pretreated with or without 10 nM ATRA for 48 hours prior to performing CDC or ADCC in the presence of 10 $\mu\text{g/ml}$ daratumumab. The Y axis shows the percent (%) CDC or ADCC. Data show the mean and SEM of at least three experiments. P-values between the indicated groups were calculated using a paired student's *t* test. Dara: daratumumab; ns: not significant.

Figure 3C shows daratumumab-induced CDC (top panel) and ADCC (bottom panel) in MM UM9 cell line pretreated with or without 10 nM ATRA for 48 hours prior to performing CDC or ADCC in the presence of 10 $\mu\text{g/ml}$ daratumumab. The Y axis shows the percent (%) CDC or ADCC. Data show the mean and SEM of at least three experiments. P-values between the indicated groups were calculated using a paired student's *t* test. Dara: daratumumab; * $p < 0.05$; ns: not significant.

Figure 4A shows that pretreatment of primary MM cells for 48 hours with 10 nM ATRA potentiates daratumumab-mediated CDC of the primary MM cells. MM cells were pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 0-10 $\mu\text{g/ml}$. The graph shows pooled results of 16 patient samples. *** $p < 0.001$, **** $p < 0.0001$. DARA: daratumumab.

Figure 4B shows that pretreatment of primary MM cells for 48 hours with 10 nM ATRA potentiates daratumumab-mediated ADCC of the primary MM cells. MM cells were pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 0-10 $\mu\text{g/ml}$. The graph shows pooled results of 13 patient samples. * $p < 0.05$. DARA: daratumumab.

Figure 5A shows the results of *in vitro* CDC of primary MM cells isolated from patient 1 and patient 2 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 5B shows the results of *in vitro* CDC of primary MM cells isolated from patient 3 and patient 4 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 5C shows the results of *in vitro* CDC of primary MM cells isolated from patient 5 and patient 6 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 5D shows the results of *in vitro* CDC of primary MM cells isolated from patient 7 and patient 8 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 5E shows the results of *in vitro* CDC of primary MM cells isolated from patient 9 and patient 10 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 5F shows the results of *in vitro* CDC of primary MM cells isolated from patient 11 and patient 12 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 5G shows the results of *in vitro* CDC of primary MM cells isolated from patient 13 and patient 14 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 5H shows the results of *in vitro* CDC of primary MM cells isolated from patient 15 and patient 16 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 6A shows the results of *in vitro* ADCC of primary MM cells isolated from patient 3 and patient 4 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 6B shows the results of *in vitro* ADCC of primary MM cells isolated from patient 7 and patient 8 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 6C shows the results of *in vitro* ADCC of primary MM cells isolated from patient 9 and patient 10 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 6D shows the results of *in vitro* ADCC of primary MM cells isolated from patient 14 and patient 15 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 6E shows the results of *in vitro* ADCC of primary MM cells isolated from patient 16 and patient 17 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 6F shows the results of *in vitro* ADCC of primary MM cells isolated from patient 18 pretreated for 48 hours with or without 10 nM ATRA as indicated in the Figure at daratumumab concentrations ranging from 1-10 µg/ml.

Figure 7 shows CD38 expression levels in BM-MNCs isolated from MM patients before and after incubation of cells with (black bars) or without (white bars) in the presence of 10 nM ATRA. The same patient samples were used in ADCC and CDC assays as shown in Figures 4A, 4B, 5 and 6.

Figure 8A shows ATRA-induced reduction of CD55, CD59 and CD46 expression on RPMI8226 cells after 48 hour incubation of cells with 0- 25 nM ATRA. MFI; mean fluorescent intensity. Expression of CD55, CD59 and CD46 were assessed using flow cytometry. Top panel: MFI; bottom panel: MFI fold change when compared to control.

Figure 8B shows ATRA-induced reduction of CD55, CD59 and CD46 expression on UM9 cells after 48 hour incubation of cells with 0- 25 nM ATRA. MFI; mean fluorescent intensity. Expression of CD55, CD59 and CD46 were assessed using flow cytometry. Top panel: MFI; bottom panel: MFI fold change when compared to control.

Figure 8C shows ATRA-induced reduction of CD55, CD59 and CD46 expression on XG1 cells after 48 hour incubation of cells with 0- 25 nM ATRA. MFI; mean fluorescent intensity. Expression of CD55, CD59 and CD46 were assessed using flow cytometry. Top panel: MFI; bottom panel: MFI fold change when compared to control.

Figure 9A shows ATRA-induced reduction of CD55 expression on primary MM cells after 48 hour incubation of cells with (grey bars) or without (black bars) in 10 nM ATRA as indicated. * p = 0.019.

Figure 9B shows ATRA-induced reduction of CD59 expression on primary MM cells after 48 hour incubation of cells with (grey bars) or without (black bars) in 10 nM ATRA as indicated. ** $p = 0.0047$.

Figure 9C shows effect of ATRA on CD46 expression on primary MM cells after 48 hour incubation of cells with (grey bars) or without (black bars) in 10 nM ATRA as indicated. ns: not significant.

Figure 10A shows CD55 expression on primary MM cells isolated from 16 MM patients after 48 hour incubation of cells with (black bars) or without (white bars) 10 nM ATRA. The same patient samples were used in CDC assays as shown in Figure 5.

Figure 10B shows CD59 expression on primary MM cells isolated from 16 MM patients after 48 hour incubation of cells with (black bars) or without (white bars) 10 nM ATRA. The same patient samples were used in CDC assays as shown in Figure 5.

Figure 10C shows CD46 expression on primary MM cells isolated from 16 MM patients after 48 hour incubation of cells with (black bars) or without (white bars) 10 nM ATRA. The same patient samples were used in CDC assays as shown in Figure 5.

Figure 11 shows that ATRA improves response to daratumumab in a humanized multiple myeloma mouse model. $Rag2^{-/-}\gamma_c^{-/-}$ mice carrying mesenchymal stem cell (MSC)-coated scaffolds were inoculated with luciferase-transduced XG1 cells. Mice were treated with control, ATRA plus T-cell depleted PBMCs as effector cells (PBMC-T), daratumumab plus PBMC-T, or daratumumab plus ATRA plus PBMC-T, and monitored weekly by bioluminescent imaging (BLI) for growth of the transduced XG1 cells. The Figure shows tumor load per treatment group with 4 mice per group and each mouse with 4 scaffolds. Statistical differences between mice treated with daratumumab and mice treated with daratumumab plus ATRA were calculated using the Mann-Whitney U-test. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$; ns: not significant.

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 the amino acid sequence shown in SEQ ID NO: 1

“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 (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

“Antibody fragments” as used herein 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 CHI domains, a F(ab)₂ 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 CHI domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody, a domain antibody (dAb) (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.

“Isolated antibody” as used herein refers to an antibody or antibody fragment that is substantially free of other antibodies having different antigenic specificities (e.g., an antibody that specifically binds CD38). An isolated antibody that specifically binds CD38, however, may 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: 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); “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](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.

“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 so that the framework may not be an exact copy of expressed human immunoglobulin or germline gene sequences.

“Human-adapted” antibodies or “human framework adapted (HFA)” antibodies refers to humanized antibodies adapted according to methods described in U.S. Pat. Publ. No. US2009/0118127. Human-adapted antibodies are humanized by selecting the acceptor human frameworks based on the maximum CDR and FR similarities, length compatibilities and sequence similarities of CDR1 and CDR2 loops and a portion of light chain CDR3 loops.

“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 where 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. A 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, for example as described in 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 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.

"Recombinant antibody" as used herein includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as antibodies isolated from an animal (e.g., a mouse or a rat) 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 such as bispecific antibodies.

"Monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity via its VH, VL and/or VH/VL pair and affinity for a particular epitope, or in a case of a bispecific monoclonal antibody, a dual binding specificity to two distinct epitopes.

"Epitope" as used herein means 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 may be composed of contiguous and/or noncontiguous amino acids that form a conformational spatial unit. For a noncontiguous 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” as used herein refers to a polypeptide or a polynucleotide that differs from a reference polypeptide or a reference polynucleotide by one or more modifications for example, substitution, insertion or deletion.

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

“In combination with” as used herein means that two or more therapeutics maybe administered to a subject together in a mixture, concurrently as single agents or sequentially as single agents in any order.

The terms “treat” or “treatment” refer to therapeutic treatment wherein the object is to slow down (lessen) an undesired physiological change or disease, or provide a beneficial or desired clinical outcome during treatment, such as the development, growth or spread of tumor or tumor cells. Beneficial or desired clinical outcomes 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. “Treatment” can also mean prolonging survival as compared to expected survival if a subject was not receiving treatment. Those in need of treatment include those subjects already with the undesired physiological change or disease as well as those subjects prone to have the physiological change or disease.

“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 may occur by a variety of mechanisms, for example by antibody-dependent cell-mediated toxicity (ADCC), antibody dependent cellular phagocytosis (ADCP), complement dependent cytotoxicity (CDC), apoptosis, necrosis, or inhibition of cell proliferation.

A “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 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.

The invention provides methods for treating patients having CD38-positive hematological malignancy with the combination of a CD38 antibody and all-trans retinoic acid (ATRA). The invention is based, at least in part, on the discovery that ATRA augments anti-CD38 antibody daratumumab-mediated lysis by ADCC and/or CDC of primary MM cells expressing low, intermediate or high levels of CD38 by enhancing CD38 expression on MM cells. ATRA is also able to induce daratumumab-mediated ADCC and/or CDC in primary MM samples which were resistant to daratumumab-mediated CDC and/or ADCC *in vitro* or were obtained from heavily pretreated multiple myeloma patients having double-refractory (lenalidomide- and bortezomib-refractory) disease. ATRA augmented daratumumab-mediated CDC to a higher extent than ADCC, which may be explained by the findings that ATRA also down-regulates complement-inhibitory proteins CD55 and CD59.

ATRA (CAS 302-79-4) has a well-known molecular structure.

One embodiment of the invention disclosed herein, including in the numbered embodiments listed below, is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA).

One embodiment of the invention disclosed herein, including in the numbered embodiments listed below, is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC).

The methods of the invention may be used to treat an animal subject belonging to any classification. Examples of such animals include mammals such as humans, rodents, dogs, cats and farm animals.

In some embodiments of the invention disclosed herein, including in the numbered embodiments listed below, the anti-CD38 antibody induces killing of the CD38-expressing cells by CDC *in vitro*.

“CD38-positive hematological malignancy” refers to a hematological malignancy characterized by the presence of tumor cells expressing CD38 including leukemias, lymphomas and myeloma. Examples of 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 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 β -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 one embodiment of the invention disclosed herein, including in the numbered embodiments listed below, the CD38-positive hematological malignancy is multiple myeloma.

In one embodiment of the invention disclosed herein, including in the numbered embodiments listed below, the CD38-positive hematological malignancy is diffuse large B-cell lymphoma (DLBCL).

In one embodiment of the invention disclosed herein, including in the numbered embodiments listed below, the CD38-positive hematological malignancy is non-Hodgkin's lymphoma.

In one embodiment of the invention disclosed herein, including in the numbered embodiments listed below, the CD38-positive hematological malignancy is acute lymphoblastic leukemia (ALL).

In one embodiment of the invention disclosed herein, including in the numbered embodiments listed below, the CD38-positive hematological malignancy is follicular lymphoma (FL).

In one embodiment of the invention disclosed herein, including in the numbered embodiments listed below, the CD38-positive hematological malignancy is Burkitt's lymphoma (BL).

In one embodiment of the invention disclosed herein, including in the numbered embodiments listed below, the CD38-positive hematological malignancy is mantle cell lymphoma (MCL).

In one embodiment of the invention disclosed herein, including in the numbered embodiments listed below, the CD38-positive hematological malignancy is multiple myeloma, acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma, diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), follicular lymphoma (FL) or mantle-cell lymphoma (MCL).

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 γ , μ , and α disease), lymphomas induced by therapy with immunosuppressive agents, such as cyclosporine-induced lymphoma, and methotrexate-induced lymphoma.

In one embodiment of the present invention, including in the numbered embodiments listed below 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, 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 as disclosed herein, including in the numbered embodiments listed below.

In some embodiments, the anti-CD38 antibody induces *in vitro* killing of CD38-expressing cells by antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC).

The variable regions of the anti-CD38 antibodies may be obtained from existing anti-CD38 antibodies, and cloned as full length antibodies or into various antibody formats and fragments using standard methods. Exemplary variable regions binding CD38 that may be used are described 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 daratumumab. Daratumumab comprises the heavy chain variable region (VH) and the light chain variable region (VL) amino acid sequences shown in SEQ ID NO: 4 and 5, respectively, heavy chain CDRs HCDR1, HCDR2 and HCDR3 of SEQ ID NOs: 6, 7 and 8, respectively, and light chain CDRs LCDR1, LCDR2 and LCDR3 of SEQ ID NOs: 9, 10 and 11, respectively, and is of IgG1/k subtype and described in U.S. Pat. No. 7,829,693. 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

MANCEFSPVSGDKPCCRLSRRAQLCLGVSVLILVVVLAVVVPRWRQQWSPGPT
TKRFPETVLARCVKYTEIHPMRHVDCQSVWDAFKGAFISKHPCNITEEDYQPLM

KLGTQTVPCNKILLWSRIKDLAQFTQVQRDMFTLEDTLGYLADDLTWCGEFN
TSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSKIFDK
NSTFGSVEVHNLQPEKVQTLLEAWVIHGGREDSRDLCQDPTIKELESII SKRNIQFSC
KNIYRPDKFLQCVKNPEDSSCTSEI

SEQ ID NO: 2

SKRNIQFSC KNIYR

SEQ ID NO: 3

EKVQTLLEAWVIHGG

SEQ ID NO: 4

EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSA
ISGSGGGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYFCAKDK
ILWFGPEPVFDYWGQGTLVTVSS

SEQ ID NO: 5

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLA WYQQKPGQAPRLLIYD
ASNRATGIPARFSGSGSGTDFTLTIS SLEPEDFAVYYCQQRSNWPPTFGQ
GTKVEIK

SEQ ID NO: 6

SFAMS

SEQ ID NO: 7

AISGSGGGTTYADSVKG

SEQ ID NO: 8

DKILWFGPEPVFDY

SEQ ID NO: 9

RASQSVSSYLA

SEQ ID NO: 10

DASNRAT

SEQ ID NO: 11

QQRSNWPPTF

SEQ ID NO: 12

EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSAISGSG
GGTTYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYFCAKDKILWFGPEPVF
DYWGQGT LVT VSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVT VSWN
SGALTSGVHTFPAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKV D KRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKV S
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTPPVLDSDGSFFLYSKLTV D KSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

SEQ ID NO: 13

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASN RAT
GIPARFSGSGSGTDFTLT ISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSPVTKSFNRGEC

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. The VH and the VL of mAb003 may be expressed as IgG1/ κ .

SEQ ID NO: 14

QVQLVQSGAEVKKPGSSVKV SCKASGGTFSSYAFSWVRQAPGQGLEWMGRVIPF
LGIANSAQKFQGRVTITADKSTSTAY
MDLSSLRSEDTAVYYCARDIAALGPFDYWGQGT LVT VSSAS

SEQ ID NO: 15

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQS
GVPSRFSGSGSGTDFTLT ISSLQP
EDFATYYCQQYNSYPRTFGQGTKVEIK

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, described in U.S. Pat. No. 7,829,693. The VH and the VL of mAb024 may be expressed as IgG1/κ.

SEQ ID NO: 16

EVQLVQSGAEVKKPGESLKISCKGSGYSFSNYWIGWVRQMPGKGLEWMGHIYPH
DSDARYSPSFQGGVTFSAADKSISTAY
LQWSSLKASDTAMYYCARHVGWGSRYWYFDLWGRGTLTVSS

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, described in US. Pat. No. 8,088,896. The VH and the VL of MOR-202 may be expressed as IgG1/κ.

SEQ ID NO: 18

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYMNWVRQAPGKGLEWVSGISGD
PSNTYYADSVKGRFTISRDNKNTLY
LQMNSLRAEDTAVYYCARDLPLVYTGFAYWGQGTLTVSS

SEQ ID NO: 19

DIELTQPPSVSVAPGQTARISCSGDNLRHYYVYWYQQKPGQAPVLVIYGDSKRPS
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

IYPGDGDTGYAQKFQ GKATLTADKSSKTVYMHLSLASEDSAVYYCARGD
 YYGSNSLDYWGQGTSVTVSS

SEQ ID NO: 21:

DIVMTQSHLSMSTSLGDPVSITCKASQDVSTVVAWYQQKPGQSPRRLIYS
 ASYRYIGVPDRFTGSGAGTDFTFITSSVQAEDLAVYYCQQHYSPPYTFGG
 GTKLEIK

Other exemplary anti-CD38 antibodies that may be used in the methods of the invention include those described in Int. Pat. Publ. No. WO05/103083, Int. Pat. Publ. No. WO06/125640, Int. Pat. Publ. No. WO07/042309, Int. Pat. Publ. No. WO08/047242 or Int. Pat. Publ. No. WO14/178820.

Anti-CD38 antibodies used in the methods of the invention disclosed herein, including in the numbered embodiments listed below, may also be selected *de novo* from 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 from for example 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.*, J. Mol. Biol. 397:385-96, 2010 and PCT Intl. Publ. No. WO09/085462). The antibody libraries may be screened for binding to human CD38 extracellular domain, obtained positive clones further characterized, 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 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). 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 the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is of IgG1, IgG2, IgG3 or IgG4 isotype.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is of IgG1 or IgG3 isotype.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody induces *in vitro* killing of CD38-expressing cells by ADCC.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody induces *in vitro* killing of CD38-expressing cells by CDC.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody induces killing of CD38-expressing cells by ADCC and CDC *in vitro*.

"Antibody-dependent cellular cytotoxicity," or "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 cells, 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 *in vitro*, 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 is generally detected by the release of label (e.g., radioactive substrates, fluorescent dyes or natural intracellular proteins) from the lysed cells. For example, primary BM-MNC cells isolated from a patient with a B-cell malignancy such as MM may be used for the assay. In an exemplary assay, BM-MNCs may be treated with an anti-CD38 antibody for 1 hour at a concentration of 0.3-10 $\mu\text{g/ml}$, and the survival of primary CD138⁺ MM cells may be determined by flow cytometry using techniques described in van der Veer *et al.*, *Haematologica* 96:284-290, 2001 or in 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 ADCC by about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of control.

“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 $\mu\text{g/ml}$, and the survival of primary CD138⁺ 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%.

The ability of monoclonal antibodies to induce ADCC may be enhanced by engineering their oligosaccharide component. Human IgG1 or IgG3 are N-glycosylated at Asn297 with the majority of the glycans in the well-known 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 Fc γ RIIIa binding without altering antigen binding or CDC activity. Such antibodies may be achieved using different methods reported to lead to the

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 277:26733-40, 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 α 1,6-fucosyltransferase (*FUT8*) gene (Mori *et al.*, Biotechnol Bioeng 88:901-908, 2004), or co-expression of β -1,4-*N*-acetylglucosaminyltransferase III and Golgi α -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). ADCC elicited by anti-CD38 antibodies used in the methods of the invention, and in some embodiments of each and every one of the numbered embodiments listed below, may also 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. CDC elicited by anti-CD38 antibodies used in the methods of the invention, and in some embodiments of each and every one of the numbered embodiments listed below, may also be enhanced by certain substitutions in the antibody Fc. Exemplary substitutions are, for example, substitutions at amino acid positions 423, 268, 267 and/or 113 (residue numbering according to the EU index) as described in Moore *et al.*, MAbs 2:181-189, 2010.

In some methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibodies comprise a substitution in the antibody Fc.

In some methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, 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 and/or 430 (residue numbering according to the EU index).

In some methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibodies comprise a substitution in the antibody Fc at amino acid position 113, 267, 268 and/or 423 (residue numbering according to the EU index).

Another embodiment of the invention, including in the numbered embodiments listed below, is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody 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 (daratumumab).

Another embodiment of the invention, including in the numbered embodiments listed below, is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC), wherein the anti-CD38 antibody 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 (daratumumab).

Antibodies may be evaluated for their competition with 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 daratumumab 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 unlabeled daratumumab 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 antibodies may be detected using horseradish peroxidase (HRP)-conjugated streptavidine and the signal detected using standard methods. It is readily apparent that in the competition assays, daratumumab may be labelled and the test antibody unlabeled. The test antibody competes with daratumumab when daratumumab inhibits binding of the test antibody, or the test antibody inhibits binding of daratumumab by 80%, 85%, 90%, 95% or 100%. The epitope of the test antibody can further be defined, for example, by peptide mapping or hydrogen/deuterium protection assays using known methods.

Another embodiment of the invention disclosed herein, including in the numbered embodiments listed below, is 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) in combination with all-trans retinoic acid (ATRA).

Another embodiment of the invention disclosed herein, including in the numbered embodiments listed below, is 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) in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC). The antibody “binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3)” when the antibody binds at least one amino acid residue within each region. The antibody may bind for example 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13 or 14 amino acid residues within each region of SEQ ID NO:2 and SEQ ID NO: 3. The antibody may also optionally bind one or more residues outside of the regions of SEQ ID NO: 2 and SEQ ID NO: 3. Binding may be assessed by known methods such as mutagenesis studies or by resolving the crystal structure of CD38 in complex with the antibody. In some embodiments disclosed herein, including in the numbered embodiments listed below, the antibody epitope comprises 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 disclosed herein, including in the numbered embodiments listed below, the antibody epitope comprises 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 disclosed herein, including in the numbered embodiments listed below, the antibody epitope comprises 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). In some embodiments disclosed herein, including in the numbered embodiments listed below, the anti-CD38 antibody binds to an epitope comprising at least KRN in the region

SKRNIQFSCCKNIYR (SEQ ID NO: 2) and comprising at least VQLT (SEQ ID NO: 22) in the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1).

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody binds to an epitope comprising at least KRN in the region SKRNIQFSCCKNIYR (SEQ ID NO: 2) and comprising at least VQLT (SEQ ID NO: 22) 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) or minimally to residues KRN and VQLT (SEQ ID NO: 22) as shown above is daratumumab having certain VH, VL and CDR sequences as described above. Antibodies that bind 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. Antibodies may be further evaluated, for example, by assaying competition between daratumumab and a test antibody for binding to CD38 as described above.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody may bind human CD38 with a range of affinities (K_D). In one embodiment according to the invention, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody binds to CD38 with high affinity, for example, with a K_D equal to or less than about 10^{-7} M, such as about 1, 2, 3, 4, 5, 6, 7, 8, or 9×10^{-8} M, 1×10^{-9} M, about 1×10^{-10} M, about 1×10^{-11} M, about 1×10^{-12} M, about 1×10^{-13} M, about 1×10^{-14} M, about 1×10^{-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. One exemplary affinity is equal to or less than 1×10^{-8} M. Another exemplary affinity is equal to or less than 1×10^{-9} M.

In some methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody has a biantennary glycan structure with fucose content of about between 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 methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, 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” refers to 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. Glycostructures 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) 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 mAb to constituent peptides by enzymatic digestion (e.g., trypsin or endopeptidase Lys-C), and subsequent separation, detection and quantitation by HPLC-MS (UPLC-MS); or 5) separation of the mAb oligosaccharides from the mAb protein by specific enzymatic deglycosylation with PNGase F at Asn 297. The oligosaccharides released may 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% - 15%.

“Normal fucose” or ‘normal fucose content’ as used herein refers to antibodies with fucose content of about over 50%, typically about over 60%, 70%, 80% or over 85%.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody 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.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody 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.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody 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 and the light chain complementarity determining regions (LCDR) 1 (LCDR1), 2 (LCDR2) and 3 (LCDR3) sequences of SEQ ID NOs: 9, 10 and 11, respectively.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, 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.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the heavy chain variable region (VH) of SEQ ID NO: 14 and the light chain variable region (VL) of SEQ ID NO: 15.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the heavy chain variable region (VH) of SEQ ID NO: 16 and the light chain variable region (VL) of SEQ ID NO: 17.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the heavy chain variable region (VH) of SEQ ID NO: 18 and the light chain variable region (VL) of SEQ ID NO: 19.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises the heavy chain variable region (VH) of SEQ ID NO: 20 and the light chain variable region (VL) of SEQ ID NO: 21.

In some methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody comprises a heavy chain comprising an amino acid sequence that is 95%, 96%, 97%, 98% or 99% identical to that of SEQ ID NO: 12 and a light chain comprising an amino acid sequence that is 95%, 96%, 97%, 98% or 99% identical to that of SEQ ID NO: 13.

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, and in some embodiments of each and every one of the numbered embodiments listed below. 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 GenomeQuestTM (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, their ability to induce ADCC, ADCP or apoptosis *in vitro* using methods described herein.

In some embodiments, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is a bispecific antibody. The VL and/or the VH regions of the existing anti-CD38 antibodies or the VL and VH regions identified *de novo* as described above may be engineered into bispecific full length antibodies. Such bispecific antibodies may be made by modulating the CH3 interactions between the two monospecific antibody heavy chains 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. Additional bispecific structures into which the VL and/or the VH regions of the antibodies of the invention can be incorporated are for example Dual Variable Domain Immunoglobulins (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).

Another embodiment of the invention is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA), wherein the CD38-positive hematological malignancy is multiple myeloma (MM), acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma, diffuse large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), follicular lymphoma (FL) or mantle-cell lymphoma (MCL).

Another embodiment of the invention is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC), wherein the CD38-positive hematological malignancy is multiple myeloma (MM), acute lymphoblastic leukemia (ALL), non-Hodgkin's lymphoma, diffuse

large B-cell lymphoma (DLBCL), Burkitt's lymphoma (BL), follicular lymphoma (FL) or mantle-cell lymphoma (MCL).

Another embodiment of the invention is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA), wherein the CD38-positive hematological malignancy is multiple myeloma (MM).

Another embodiment of the invention is a method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 antibody in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC), wherein the CD38-positive hematological malignancy is multiple myeloma (MM).

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 in combination with all-trans retinoic acid (ATRA), wherein the subject is resistant to or has acquired resistance to treatment with the anti-CD38 antibody.

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 in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC), wherein the subject is resistant to or has acquired resistance to treatment with the anti-CD38 antibody.

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 in combination with all-trans retinoic acid (ATRA), wherein the subject is resistant to or has acquired resistance to treatment with at least one chemotherapeutic agent.

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 in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity

(CDC), wherein the subject is resistant to or has acquired resistance to treatment with at least one chemotherapeutic agent.

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 in combination with all-trans retinoic acid (ATRA), wherein the subject is resistant to or has acquired resistance to treatment with at least one chemotherapeutic agent.

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 in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC), wherein the subject is resistant to or has acquired resistance to treatment with at least one chemotherapeutic agent.

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject is resistant to or has acquired resistance to treatment with at least one chemotherapeutic agent, wherein the at least one chemotherapeutic agent is lenalidomide, bortezomib, melphalan, dexamethasone or thalidomide.

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject is resistant to or has acquired resistance to treatment with at least one chemotherapeutic agent, wherein the at least one chemotherapeutic agent is lenalidomide, bortezomib, melphalan, dexamethasone, thalidomide, cyclophosphamide, hydroxydaunorubicin (doxorubicin), vincristine or prednisone.

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject is resistant to or has acquired resistance to treatment with at least one chemotherapeutic agent, wherein the at least one chemotherapeutic agent is lenalidomide and/or bortezomib.

Various qualitative and/or quantitative methods may be used to determine if a subject is resistant, has developed or is susceptible to developing a resistance to treatment with an anti-CD38 antibody or other therapeutic agent. Symptoms that may be associated with resistance include, for example, a decline or plateau of the well-being of the patient, an increase in the size of a tumor, increase in the number of cancer cells, arrested or slowed decline in growth of a tumor or tumor cells, and/or the spread of cancerous cells in the body from one location to other organs, tissues or cells. Re-establishment or

worsening of various symptoms associated with tumor may also be an indication that a subject has developed or is susceptible to developing resistance to an anti-CD38 antibody or other therapeutic agent. The symptoms associated with cancer may vary according to the type of cancer. For example, symptoms associated with B-cell malignancies may include swollen lymph nodes in neck, groin or armpits, fever, night sweats, coughing, chest pain, unexplained weight loss, abdominal swelling or pain, or a feeling of fullness. Remission in malignant lymphomas is standardized using the Cheson criteria (Cheson *et al.*, J Clin Oncology 25:579-586, 2007), which guidelines can be used to determine if a subject has developed a resistance to an anti-CD38 antibody or other therapeutic agent.

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject having a CD38-positive hematological malignancy is homozygous for phenylalanine at position 158 of CD16 (FcγRIIIa-158F/F genotype) or heterozygous for valine and pheynylalanine 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.

The invention also provides for the method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 in combination with all-trans retinoic acid (ATRA), wherein the subject is homozygous for phenylalanine at position 158 of CD16 or heterozygous for valine and pheynylalanine at position 158 of CD16.

The invention also provides for the method of treating a subject having a CD38-positive hematological malignancy, comprising administering to the subject in need thereof an anti-CD38 in combination with all-trans retinoic acid (ATRA), wherein the anti-CD38 antibody induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity

(CDC), wherein the subject is homozygous for phenylalanine at position 158 of CD16 or heterozygous for valine and pheynylalanine at position 158 of CD16.

Administration/ Pharmaceutical Compositions

In the methods of the invention, and in some embodiments of each and every one of the numbered embodiments listed below, 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 may 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, 21st 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, and in some embodiments of each and every one of the numbered embodiments listed below, may be administered to a patient by any suitable route, for example parentally by intravenous (*i.v.*) infusion or bolus injection, intramuscularly or subcutaneously or intraperitoneally. *i.v.* infusion may 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 a 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/kg to about 100 mg/kg, e.g. about 0.05 mg/kg 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, e.g., 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/m². Usually between 1 and 8 doses, (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) may be administered to treat a CD38-positive B-cell malignancy, 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 and in some embodiments of each and every one of the numbered embodiments listed below, 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 and in some embodiments of each and every one of the numbered embodiments listed below, 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 and in some embodiments of each and every one of the numbered embodiments listed below, 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 and in some embodiments of each and every one of the numbered embodiments listed below, 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 and in some embodiments of each and every one of the numbered embodiments listed below, 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.

The anti-CD38 antibody in the methods of the invention and in some embodiments of each and every one of the numbered embodiments listed below may be administered in combination with all-trans retinoic acid (ATRA).

ATRA may be provided as a dosage of 45 mg/m²/day PO or 25 mg/m²/day PO.

The anti-CD38 antibody in the methods of the invention and in some embodiments of each and every one of the numbered embodiments listed below may be administered in combination with all-trans retinoic acid (ATRA) and a third therapeutic agent.

In the methods of the invention, and in some embodiments of each and every one of the numbered embodiments listed below, the third therapeutic agent may be melphalan, mechlorethamine, thioepa, chlorambucil, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, dacarbazine (DTIC), procarbazine, mitomycin C, cisplatin and other platinum derivatives, such as carboplatin, thalidomide or a thalidomide analog, lenalidomide or CC4047, a proteasome inhibitor, such as bortezomib or vinca alkaloid, such as vincristine or an anthracycline, such as doxorubicin.

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.

Further embodiments of the invention

Set out below are certain further embodiments of the invention according to the disclosures elsewhere herein. Features from embodiments of the invention set out above described as relating to the invention disclosed herein also relate to each and every one of these further numbered embodiments.

1. An anti-CD38 antibody for use in treating a subject having a CD38-positive hematological malignancy, in combination with all-trans retinoic acid (ATRA).
2. ATRA 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 ATRA for use in treating a subject having a CD38-positive hematological malignancy.
4. The anti-CD38 antibody for use according to embodiment 1, ATRA for use according to embodiment 2, or the combination for use according to embodiment 3, wherein the anti-CD38 antibody induces killing of the CD38-expressing cells by
 - a. antibody-dependent cell-mediated cytotoxicity (ADCC);
 - b. complement dependent cytotoxicity (CDC); or
 - c. both ADCC and CDC *in vitro*.
5. The anti-CD38 antibody for use according to embodiment 1, ATRA for use according to embodiment 2, or the combination for use according to embodiment 3, wherein the anti-CD38 antibody induces killing of the CD38-expressing cells by ADCC *in vitro*.
6. The anti-CD38 antibody for use according to embodiment 1, 4 or 5, ATRA for use according to embodiment 2, 4 or 5, or the combination for use according to embodiment 3-5, 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) or mantle-cell lymphoma (MCL).
7. The anti-CD38 antibody for use according to embodiment 1, 4-6, ATRA for use according to embodiment 2, 4-6, or the combination for use according to embodiment 3-6, wherein the CD38-positive hematological malignancy is MM.
8. The anti-CD38 antibody for use according to embodiment 1, 4-7, ATRA for use according to embodiment 2, 4-7, or the combination for use according to embodiment 3-7, wherein the subject is resistant to or has acquired resistance to

treatment with at least one chemotherapeutic agent, and anti-CD38 antibody, or a combination of at least one chemotherapeutic agent and an anti-CD38 antibody.

- 9.** The anti-CD38 antibody for use according to embodiment 1, 4-8, ATRA for use according to embodiment 2, 4-8, or the combination for use according to embodiment 3-8, wherein the at least one chemotherapeutic agent is lenalidomide, bortezomib, melphalan, dexamethasone or thalidomide.
- 10.** The anti-CD38 antibody for use according to embodiment 1, 4-9, ATRA for use according to embodiment 2, 4-9, or the combination for use according to embodiment 3-9, wherein the at least one chemotherapeutic agent is lenalidomide or bortezomib.
- 11.** The anti-CD38 antibody for use according to embodiment 1, 4-10, ATRA for use according to embodiment 2, 4-10, or the combination for use according to embodiment 3-10, wherein
 - a. the anti-CD38 antibody is of IgG1, IgG2, IgG3 or IgG4 isotype;
 - b. the anti-CD38 antibody 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;
 - c. the anti-CD38 antibody binds to the region SKRNIQFSCKNYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1);
 - d. the anti-CD38 antibody 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;
 - e. the anti-CD38 antibody 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;
 - f. 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;
 - g. the anti-CD38 antibody comprises a heavy chain comprising an amino acid sequence that is 95%, 96%, 97%, 98% or 99% identical to that of SEQ ID NO: 12 and a light chain comprising an amino acid sequence that is 95%, 96%, 97%, 98% or 99% identical to that of SEQ ID NO: 13;
 - h. the anti-CD38 antibody comprises the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13

- i. the anti-CD38 antibody comprises th VH of SEQ ID NO: 14 and the VL of SEQ ID NO: 15;.
- j. the anti-CD38 antibody comprises th VH of SEQ ID NO: 16 and the VL of SEQ ID NO: 17;
- k. the anti-CD38 antibody comprises th VH of SEQ ID NO: 18 and the VL of SEQ ID NO: 19; or
- l. the anti-CD38 antibody comprises th VH of SEQ ID NO: 20 and the VL of SEQ ID NO: 21.
- m.

Example 1. General methods

Antibodies and reagents

A human mAb against an innocuous antigen (HIV-1 gp120) was used as an isotype control as described previously (van der Veers *et al.*, Haematologica 96:284-290, 2011; van der Veers *et al.*, Blood Cancer J 1:e41, 2011). All-*trans* retinoic acid (ATRA) was purchased from Sigma-Aldrich and diluted in DMSO.

Bioluminescence imaging (BLI)-based ADCC assays using luciferase (LUC)-transduced MM cell lines

LUC-transduced MM cell lines were co-cultured with effector cells (freshly isolated PBMCs from healthy donors) at an effector to target ratio of 1:25 in white opaque 96-well flat bottom plates (Costar) in the presence of daratumumab (0.001, 0.01, 0.1, and 1.0 µg/mL) for four hours. The survival of LUC⁺-MM cells was then determined by BLI, 10 minutes after addition of the substrate luciferin (125 µg/mL; Promega). Lysis of MM cells was determined using the following formula: % lysis = 1 - (mean BLI signal in the presence of effector cells and daratumumab / mean BLI signal in the presence of effector cells and control antibody) x100%.

BLI-based CDC assays using LUC-transduced MM cell lines

Daratumumab (0, 0.03, 0.1, 0.3, 1.0 and 3.0 µg/mL) was added to MM cell lines in medium supplemented with pooled human serum (10%; Sanquin) or heat-inactivated human serum. After a 1-hour incubation at 37 °C, cell lysis was determined by BLI, 10 minutes after addition of luciferin (125 µg/ml), and calculated using the following

formula: % lysis = $1 - (\text{mean BLI signal in the presence of native human serum} / \text{mean BLI signal in the presence of heat-inactivated serum}) \times 100\%$.

Flow cytometry-based *ex vivo* ADCC and CDC assays in BM-MNC

Freshly isolated BM-MNCs, containing 2-57% malignant plasma cells as determined by flow cytometry, were immediately used in *ex vivo* experiments. For ADCC experiments, BM-MNCs, containing the malignant plasma cells, as well as the patient's own effector cells, were incubated in RPMI + 10% fetal bovine serum with daratumumab (0.01–10 µg/mL) in 96-well flat-bottom plates in fully humidified incubators at 37°C, 5% CO₂-air mixture for 48 h. Sample viability at incubation was more than 98%, as assessed by using ToPro-3 (Invitrogen/Molecular Probes). For CDC assays, BM-MNCs were treated with daratumumab (0.3–10 µg/mL) and complement for 1 hour prior to flow cytometric analysis. Pooled human serum (10%) was used as a source of complement. The survival of primary CD138⁺ MM cells in the BM-MNCs was determined by flow-cytometry as previously described (van der Veers et al., *Haematologica* 96:284-290, 2011; van der Veers et al., *Blood Cancer J* 1:e41, 2011). Surviving MM cells were enumerated by single platform flow-cytometric analysis of CD138⁺ cells (with CD138-PE (Beckman Coulter, Miami, FL, USA)) in the presence of Flow-Count Fluorospheres (Beckman Coulter) to determine absolute numbers of cells. The percentage of MM cell lysis in the different treated conditions was determined relative to MM survival of wells treated with the control antibody (IgG1-b12 as IgG1 control antibody for daratumumab) using the following formula: % lysis cells = $1 - (\text{absolute number of surviving CD138}^+ \text{ cells in treated wells} / \text{absolute number of surviving CD138}^+ \text{ cells in control wells}) \times 100\%$.

Immunophenotyping by flow cytometry

Expression of several cell surface proteins was determined by flow cytometric analysis using FITC-, PE-, Per-CP-, or APC-conjugated monoclonal antibodies. Anti-CD38, anti-CD138, and anti-CD56 were purchased from Beckman Coulter; anti-CD3, anti-CD16, anti-CD55, anti-CD59 from BD Biosciences; and anti-CD46 from Biolegend. Flow cytometry was done using a FACS-Calibur device (Becton Dickinson); the data were analyzed using the CellQuest software.

Statistics

Statistical analyses were performed using Prism software (Graphpad Software Inc, version 5). Comparisons between variables were performed using two-tailed paired Student's *t* test. Correlations between variables were made using the Spearman's rank correlation coefficient. *p*-values below 0.05 were considered significant.

Example 2. ATRA increases CD38 expression on MM cell lines and in primary MM cells

An increase in CD38 expression levels may enhance the efficacy of daratumumab to kill MM cells via ADCC or CDC. Interaction of ATRA with nuclear retinoic acid receptors results in altered expression of target genes including induction of CD38 expression (Malavasi F. J Leukoc Biol 90:217-219, 2011; Drach *et al.*, Cancer Res 54:1746-1752, 1994). Therefore, effect of ATRA on MM cell lines RPMI8226, UM9, and XG1 was studied. MM cells were incubated with RPMI-1640 medium alone or with ATRA ranging from 0 – 25 nM for 48 hours (Figure 1A) or were incubated with 10 nM ATRA for 24, 48, 72 or 96 hours (Figure 1B) and then harvested to determine CD38 expression by flow cytometry using a FACS-Calibur device (Becton Dickinson) and anti-CD38 antibody (Beckman Coulter). The data were analyzed using the CellQuest software.

Minimum of 10 nM ATRA was sufficient to induce a 1.9– 4.4-fold increase in CD38 expression on the MM cell lines RPMI8226, UM9, and XG1. Higher doses of ATRA did not further enhance CD38 expression (Figure 1A). Maximum enhancement of CD38 expression occurred at 48 hours (Figure 1B). Therefore 10 nM ATRA for 48 hours was used in all subsequent experiments.

Ex vivo ATRA exposure (10 nM, 48 hours) of primary MM cells from 26 patients was also studied. In these experiments, BM-MNCs from 26 MM patients were incubated with RPMI-1640 medium alone or with 10 nM ATRA for 48 hours, incubated at 4°C for 20 min with FITC-conjugated CD38 antibody (Beckman Coulter) and then harvested to determine CD38 expression by flow cytometry. Flow cytometric analyses were performed using a FACS-Calibur device (Becton Dickinson); the data were analyzed using the CellQuest software.

ATRA induced CD38 expression (median increase 1.7-fold, range 1.0 – 26.5-fold) (Figure 2). There was also a significant upregulation of CD138 expression levels (median increase: 2.0-fold), which is characteristic of MM cell differentiation. In contrast, no significant changes in the expression of other plasma cell antigens, such as HLA A/B/C or CD56 were observed in response to ATRA.

Example 3. ATRA-mediated upregulation of CD38 enhances both daratumumab-mediated ADCC and CDC against MM cells

Possible effect of ATRA-induced upregulation of CD38 expression on daratumumab-induced ADCC and CDC was tested in MM cell lines XG-1, RPMI8226 and UM9 and in primary MM cells.

For MM cell lines, CDC and ADCC were assessed using bioluminescence imaging (BLI) based ADCC and CDC assays as described above. For primary MM cells, CDC and ADCC were assessed using Flow cytometry-based *ex vivo* ADCC and CDC assays in BM-MNC as described above. In the assays, cells were pre-treated with 10 nM ATRA or solvent control for 48 hours, followed by incubation with or without daratumumab in the presence of PBMCs as effector cells for assessment of ADCC or in the presence of human serum as complement source for analysis of CDC. Isotype control was added at 10 µg/ml, and 10% heat-inactivated serum was used as control for CDC.

Figure 3A, Figure 3B and Figure 3C show the results of daratumumab-induced CDC and ADCC in the XG1, RPMI8226 and UM9 cell lines, respectively.

10 nM ATRA alone induced no MM cell lysis. Pretreatment of MM cell lines with 10 nM ATRA significantly increased daratumumab-mediated CDC in XG-1 cells (Figure 3A), and ADCC in XG-1 (Figure 3A) and UM9 (Figure 3C) cells, compared with solvent control (Figure 3A). In RPMI8226 cells there was no significant improvement in daratumumab-mediated ADCC and CDC. These differences in ATRA responsiveness may be partly explained by the fact that ATRA enhanced CD38 expression 2.9-fold in XG-1 and 4.4-fold in UM9, while the upregulation was only 1.9-fold in RPMI8226 cells (Figure 1A and 1B).

Example 4. ATRA-mediated upregulation of CD38 enhances both daratumumab-mediated ADCC and CDC against primary MM cells.

Primary MM cells were evaluated to further explore the effect of ATRA-mediated induction of CD38 expression on daratumumab sensitivity.

Figure 4A and Figure 4B show results of daratumumab-induced CDC and ADCC, respectively, in primary MM cells pretreated for 48 hours with or without 10 nM ATRA. The graphs in Figure 4A and Figure 4B represent pooled results of 16 or 13 patient samples, respectively.

In primary MM cells, pretreatment with ATRA for 48 hours resulted in a significant increase in their susceptibility to daratumumab-mediated CDC in 13 out of 16 patients (data not shown) and ADCC in 8 out of 11 patients (data not shown). Pooled

results of these patients show that ATRA improved CDC mediated by 10 µg/mL daratumumab median from 16.1 % to 43.9 % ($P < 0.0001$) (Figure 4A), and ADCC mediated by 10 µg/mL daratumumab improved median from 25.1 % to 39.5 % ($P = 0.0315$) by ATRA (Figure 4B).

Figure 5 shows results of daratumumab-induced CDC in primary MM cells from each patient. Figure 5A shows daratumumab-induced CDC in primary MM cells from patient 1 and patient 2. Figure 5B shows daratumumab-induced CDC in primary MM cells from patient 3 and patient 4. Figure 5C shows daratumumab-induced CDC in primary MM cells from patient 5 and patient 6. Figure 5D shows daratumumab-induced CDC in primary MM cells from patient 7 and patient 8. Figure 5E shows daratumumab-induced CDC in primary MM cells from patient 9 and patient 10. Figure 5F shows daratumumab-induced CDC in primary MM cells from patient 11 and patient 12. Figure 5G shows daratumumab-induced CDC in primary MM cells from patient 13 and patient 14. Figure 5h shows daratumumab-induced CDC in primary MM cells from patient 15 and patient 16. ATRA induced daratumumab-mediated CDC in primary MM cells that were not responsive to daratumumab alone *in vitro* (for example patients 1, 4, 8, 12, 13, 15 and 16). These primary MM cells were isolated from patients with refractory or double refractory disease as indicated in Table 1. In some patient primary MM cell samples, ATRA had no additional effect enhancing daratumumab-mediated CDC (for example see patients 6, 7 and 14).

Figure 6 shows results of daratumumab-induced ADCC in primary MM cells from each patient. Figure 6A shows daratumumab-induced CDC in primary MM cells from patient 3 and patient 4. Figure 6B shows daratumumab-induced CDC in primary MM cells from patient 7 and patient 8. Figure 6C shows daratumumab-induced CDC in primary MM cells from patient 9 and patient 10. Figure 6D shows daratumumab-induced CDC in primary MM cells from patient 14 and patient 15. Figure 6E shows daratumumab-induced CDC in primary MM cells from patient 16 and patient 17. Figure 6f shows daratumumab-induced CDC in primary MM cells from patient 18. ATRA induced daratumumab-mediated ADCC most primary MM cells tested. These primary MM cells were isolated from patients with refractory or double refractory disease as indicated in Table 1.

Surface expression of CD38 was also assessed in all these tested primary MM cells in BM-MNCs incubated with RPMI-1640 medium alone or with ATRA 10 nM for 48 hours (Figure 7).

Overall the results suggest that ATRA is an attractive strategy to improve CD38 expression and daratumumab activity in MM cell lines and in primary MM cells, including MM cells that are refractory to daratumumab-mediated CDC and/or ADCC.

Table 1 shows the baseline characteristics of the BM-MNC of the tested 19 MM patients. In the table, * lenalidomide- and/or bortezomib-refractory disease is defined as progressive disease on lenalidomide - and bortezomib -therapy, no response (less than partial response) to lenalidomide - and bortezomib -therapy, or progressive disease within 60 days of stopping a lenalidomide - and bortezomib -containing regimen, according to the International Uniform Response Criteria for Multiple Myeloma.

Table 1.

	Patient					
Parameter:	1	2	3	4	5	6
Age (years)	71	43	71	64	64	55
Sex	M	M	F	M	M	F
Type of monoclonal heavy chain	IgG	-	-	IgD	-	IgG
Type of light chain	K	K	L	K	L	L
Previous therapy						
Prior lines of therapy (number)	10	4	4	6	3	0
Prior stem cell transplantation	yes	yes	yes	yes	yes	no
Autologous	yes	yes	yes	yes	yes	no
Allogeneic	no	no	no	no	no	no
Prior lenalidomide treatment,	yes	yes	yes	yes	yes	no
lenalidomide refractory status*	yes	yes	yes	yes	yes	no
Prior bortezomib treatment	yes	yes	yes	yes	yes	no
bortezomib refractory status*	yes	yes	yes	yes	yes	no
CD38 expression on MM cells (MFI)	1258	1346	764	1275	2642	1134
CD46 expression on MM cells (MFI)	1165	264	866	1346	661	1124
CD55 expression on MM cells (MFI)	610	119	552	227	1	594
CD59 expression on MM cells (MFI)	235	62	228	108	7	90

Patient						
Parameter:	7	8	9	10	11	12
Age (years)	55	64	75	63	56	59
Sex	F	M	M	F	M	M
Type of monoclonal heavy chain	IgA	-	-	IgA	IgA	-
Type of light chain	L	K	L	K	K	K
Previous therapy						
Prior lines of therapy (number)	2	2	5	6	2	4
Prior stem cell transplantation	yes	yes	no	yes	yes	yes
Autologous	yes	yes	no	yes	yes	yes
Allogeneic	no	no	no	no	no	no
Prior lenalidomide treatment,	no	yes	yes	yes	yes	yes
lenalidomide refractory status*	no	yes	yes	yes	no	yes
Prior bortezomib treatment	yes	yes	yes	yes	no	yes
bortezomib refractory status*	yes	no	yes	yes	no	no
CD38 expression on MM cells (MFI)	1999	578	1252	1310	843	64
CD46 expression on MM cells (MFI)	2288	4870	1700	196	368	264
CD55 expression on MM cells (MFI)	655	528	813	4	362	60
CD59 expression on MM cells (MFI)	92	151	241	7	74	47

Patient						
Parameter:	13	14	15	16	17	18
Age (years)	71	72	67	64	63	53
Sex	F	M	M	M	M	M
Type of monoclonal heavy chain	-	-	IgG	-	IgG	IgA
Type of light chain	L	K	K	K	L	K
Previous therapy						
Prior lines of therapy (number)	4	5	2	3	4	2
Prior stem cell transplantation	yes	no	no	yes	yes	yes
Autologous	yes	no	no	yes	yes	yes
Allogeneic	no	no	no	no	no	no

Prior lenalidomide treatment,	yes	yes	yes	yes	no	yes
lenalidomide refractory status*	yes	yes	yes	yes	no	yes
Prior bortezomib treatment	yes	yes	yes	yes	yes	yes
bortezomib refractory status*	yes	yes	no	yes	no	yes
CD38 expression on MM cells (MFI)	173	241	78	1000	667	11
CD46 expression on MM cells (MFI)	300	492	362	491	538	557
CD55 expression on MM cells (MFI)	379	1275	59	176	231	519
CD59 expression on MM cells (MFI)	188	75	9	107	70	52

BM-MNCs; bone marrow mononuclear cells. MM; multiple myeloma. M; male. F; female. K; kappa. L; lambda

Example 5. ATRA downregulates CD55 and CD59 expression in primary MM cells

The experiments conducted revealed that the pretreatment of MM cells with ATRA rendered these cells more susceptible to daratumumab-mediated ADCC and CDC. The improvement in CDC was more pronounced than the enhancement of ADCC. The molecular basis for the observation was assessed.

The effect of ATRA on effector cells was evaluated. ATRA had no effect or minimal effect on the ability of PBMCs from healthy donors to induce ADCC on human MM cell lines L363-CD38, LME-1, RPMI8226 and UM9 (data not shown). On the contrary, ATRA reduced expression levels of complement-inhibitory proteins CD55, CD59 and CD46 on MM cell lines and primary MM cells. In RPMI8226 (Figure 8A), L363 (Figure 8B) and XG-1 (Figure 8C) cells, ATRA reduced expression levels of CD55, CD59, and CD46. In primary MM cells derived from 16 patients, ATRA significantly reduced the expression of CD55 (mean reduction 21.3 %, $P = 0.019$) (Figure 9A) and CD59 (mean reduction 37.5 %, $P = 0.0047$) (Figure 9B), while ATRA did not significantly affect CD46 expression levels (data not shown). The CD46, CD55 and CD59 expression levels from the tested 16 patients' samples are shown in Figures 10A (CD55), Figure 10B (CD59) and Figure 10C (CD46). In the experiments, cells were cultured at 37°C with RPMI-1640 medium with or without 10 nM ATRA 10 nM for 48 h. Cells were then incubated at 4°C for 20 min with the appropriate conjugated antibodies panel. Flow cytometric analyses were performed using a FACS-Calibur device (Becton Dickinson); the data were analyzed using the CellQuest software.

Example 6. *In vivo* efficacy of the combination of ATRA and daratumumab against MM tumors growing in a humanized microenvironment.

Hybrid scaffolds consisting of three 2-3 mm biphasic calcium phosphate particles were coated *in vitro* with human mesenchymal stromal cells (MSCs; 2×10^5 cells/scaffold). After a week of *in vitro* culture in an osteogenic medium, humanized scaffolds were implanted subcutaneously into RAG2^{-/-} γ c^{-/-} mice, as described previously (Groen *et al.*, Blood. 19;120:e9-e16, 2012; de Haart *et al.*, Clin.Cancer Res. 19:5591-5601, 2013).

Eight weeks after implantation, mice received a sublethal irradiation dose (3 Gy, 200 kV, 4 mA) and luciferase-transduced XG1 cells were injected directly into the scaffold (1×10^6 cells/scaffold). Three weeks after inoculation, when there was visible tumor growth in the scaffolds by bioluminescent imaging (BLI), different groups of mice were treated with 1) vehicle, 2) ATRA plus T-cell depleted PBMC as effector cells (PBMC-T), 3) daratumumab plus PBMC-T, and 4) daratumumab plus ATRA plus PBMC-T. Daratumumab (8 mg/kg) was given intraperitoneally on days 23, 30, and 37; PBMC-T (8×10^6 cells/mouse) were given intravenously on days 24, 31, and 38; and ATRA (10 mg/kg) was given via intraperitoneal injection on days 21-24, 28-31, and 35-38. PBMC-T were prepared by Ficoll-Hypaque density-gradient centrifugation of buffy coats, and subsequent depletion of T cells by CD3-beads using the EasySep™-technology (STEMCELL Technologies). Tumor growth was monitored by weekly BLI measurements as described previously (Groen *et al.*, Blood. 19;120:e9-e16, 2012). All animal experiments were conducted after acquiring permission from the local ethical committee for animal experimentation and were in compliance with the Dutch Animal Experimentation Act. The statistical differences between the different treatment groups in the mice experiments were calculated using a Mann-Whitney test. *P*-values below 0.05 were considered significant.

Luciferase-transduced XG1 multiple myeloma cells developed into aggressive tumors in immunodeficient RAG2^{-/-} γ c^{-/-} mice in a humanized bone marrow microenvironment generated by subcutaneous implantation of MSC-coated ceramic scaffolds. To optimally evaluate the effects of daratumumab and ATRA, mice were co-injected with NK cell-enriched (T cell-depleted) PBMCs of a healthy donor in combination with daratumumab and/or ATRA, as RAG2^{-/-} γ c^{-/-} mice are devoid of NK cells. To follow the outgrowth of the tumor, BLI was performed weekly for 5 weeks. As shown in Figure 11, daratumumab markedly slowed tumor progression, whereas ATRA as

single agent had no effect. ATRA also significantly enhanced the anti-MM effect of daratumumab in this model.

What is claimed is:

1. The use of an anti-CD38 antibody and an all-trans retinoic acid (ATRA) in the manufacture of a medicament for the treatment of multiple myeloma in a subject, wherein the anti-CD38 antibody:
 - a) comprises heavy chain complementarity determining regions 1 (HCDR1), HCDR2 and HCDR3 amino acid sequences of SEQ ID NOs: 6, 7 and 8, respectively;
 - b) comprises light chain complementarity determining regions 1 (LCDR1), LCDR2 and LCDR3 amino acid sequences of SEQ ID NOs: 9, 10 and 11, respectively; and
 - c) induces killing of CD38-expressing cells *in vitro* by antibody-dependent cell-mediated cytotoxicity (ADCC) or complement dependent cytotoxicity (CDC).
2. The use of claim 1, wherein the anti-CD38 antibody induces killing of the CD38-expressing cells by CDC *in vitro*.
3. The use of claim 1, wherein the anti-CD38 antibody induces killing of the CD38-expressing cells by ADCC *in vitro*.
4. The use of claim 1, 2 or 3, wherein the subject is resistant to or has acquired resistance to treatment with at least one chemotherapeutic agent, the anti-CD38 antibody, or a combination of at least one chemotherapeutic agent and the anti-CD38 antibody.
5. The use of claim 4, wherein the at least one chemotherapeutic agent is lenalidomide, bortezomib, melphalan, dexamethasone or thalidomide.
6. The use of claim 5, wherein the at least one chemotherapeutic agent is lenalidomide or bortezomib.
7. The use of any one of claims 1, 2, 3 or 4-6, wherein the anti-CD38 antibody is of IgG1, IgG2, IgG3 or IgG4 isotype.
8. The use of claim 7, wherein the anti-CD38 antibody is of IgG1 isotype.

9. The use of any one of claims 1, 2, 3 or 4-8, wherein the anti-CD38 antibody comprises a heavy chain variable region (VH) of SEQ ID NO: 4 and a light chain variable region (VL) of SEQ ID NO: 5.
10. The use of any one of claims 1, 2, 3 or 4-9, wherein the anti-CD38 antibody comprises the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13.