ANTI-CD38 ANTIBODIES FOR TREATMENT OF ACUTE MYELOID LEUKEMIA

FIELD OF THE INVENTION

The present invention relates to methods of treatment of acute myeloid leukemia with anti-CD38 antibodies.

BACKGROUND OF THE INVENTION

CD38 is a type II membrane protein with ADP ribosyl cyclase activity, catalyzing formation of second messengers cyclic ADP-ribose (cADPR) and nicotinic acid adenine dinucleotide phosphate (NAADP) from NAD and NADP, respectively. CD38 mediates calcium mobilization and regulates intracellular NAD levels, and is implicated having role in various physiological functions (Funaro *et al.,* J Immunology 145:2390-6, 1990; Terhorst *et al.,* Cell 771-80, 1981; Guse *et al.,* Nature 398:70-3, 1999; Adriouch *et al.,* 14:1284-92, 2012; Chiarugi *et al.,* Nature Reviews 12:741-52, 2012; Wei *et al.,* WJBC 5:58-67, 2014)

Acute myeloid leukemia (AML) is a heterogeneous hematologic disorder characterized by clonal expansion of myeloid blasts in bone marrow, peripheral blood and other tissues. Despite recent progress, current treatment of AML remains unsatisfactory with a 5-year relapse-free survival rate lower than 30%.

Therefore, there remains a need for effective treatments for AML.

SUMMARY OF THE INVENTION

One embodiment of the invention is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody for a time sufficient to treat AML.

One embodiment of the invention is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody that 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 for a time sufficient to treat AML.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows daratumumab-induced apoptosis in the absence of crosslinking in NB-4 AML cell line. PI: propidium iodide.

Figure 1B shows daratumumab-induced apoptosis in the presence of crosslinking in NB-4 AML cell line. PI: propidium iodide.

Figure 2A shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 3406 model as measured by reduction in percentage (%) leukemic $CD45^+CD33^+$ cells in bone marrow (BM), spleen (SPL) and peripheral blood (PB). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p values are indicated in the Figure (isotype control vs. daratumumab).

Figure 2B shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 7577 model as measured by reduction in percentage (%) leukemic $CD45^+CD33^+$ cells in bone marrow (BM), spleen (SPL) and peripheral blood (PB). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. ns: not significant. ***$p<0.001$

Figure 2C shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 8096 model, assessed by reduction in percentage (%) leukemic $CD45^+CD33^+$ cells in bone marrow (BM), spleen (SPL) and peripheral blood (PB). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. ns: not significant. *$p<0.05$

Figure 3A shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 3406 model, assessed by reduction in total leukemic burden in bone marrow (number of $CD45^+CD33^+$ cells per four bones). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. There was no significant difference ($p>0.01$) in bone marrow leukemic burden between Ctrl and Dara. p value between isotype control vs daratumumab treatment groups shown.

Figure 3B shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 3406 model, assessed by reduction in total leukemic burden in spleen (number of $CD45^+CD33^+$ cells per spleen). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p value between isotype control vs daratumumab treatment groups shown.

Figure 3C shows the efficacy of daratumumab in patient-derived xenograft (PDX) AML 3406 model, assessed by reduction in total leukemic burden in peripheral blood (number of $CD45^+CD33^+$ cells per μl blood). Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p value between isotype control vs daratumumab treatment groups is indicated.

Figure 4A shows daratumumab-induced downregulation of surface CD38 expression in patient-derived xenograft (PDX) AML 3406 model in bone marrow (BM), spleen (SPL) and peripheral blood (PB) after 5 weeks of treatment with daratumumab. Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p values as indicated in the Figure for isotype control vs. daratumumab.

Figure 4B shows daratumumab-induced reduction in the percentage of CD38-positive leukemia blasts in patient-derived xenograft (PDX) AML 3406 model in bone marrow (BM), spleen (SPL) and peripheral blood (PB) after 5 weeks of treatment with daratumumab. Ctrl: no treatment; IgG1: isotype control; Dara: daratumumab. p values are indicated in between isotype control vs. daratumumab treatment groups.

Figure 5A shows the efficacy of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) in reducing leukemia burden in patient-derived xenograft (PDX) 3406 model in bone marrow. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<.001. ns: not significant.

Figure 5B shows the efficacy of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) in reducing leukemia burden in patient-derived xenograft (PDX) 3406 model in spleen. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

Figure 5C shows the efficacy of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) in reducing leukemia burden in patient-derived xenograft (PDX) model in peripheral blood. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

Figure 6A shows the effect of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) on CD38 expression on $CD45^+CD33^+$ AML bone marrow blasts in patient derived xenograft (PDX) 3406 model. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant. MFI: mean fluorescent intensity.

Figure 6B shows the effect of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) on CD38 expression on $CD45^+CD33^+$ AML spleen blasts in patient derived xenograft (PDX) 3406 model. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *p<0.05; **p<0.01; ***p<0.001. ns: not significant.

Figure 6C shows the effect of daratumumab (dara) alone or in combination with dacogen (DAC) or cytrabine and doxorubicin (chemo) on CD38 expression on $CD45^+CD33^+$ AML peripheral blood blasts in patient derived xenograft (PDX) 3406 model. Leukemia burden was assessed as % of $CD45^+CD33^+$ cells. Ctrl: isotype control. *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 an 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 may 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 IgA_1, IgA_2, IgG_1, IgG_2, IgG_3 and IgG_4. Antibody light chains of any vertebrate species may 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" refers to a portion of an immunoglobulin molecule that retains the heavy chain and/or the light chain antigen binding site, such as heavy chain complementarity determining regions (HCDR) 1, 2 and 3, light chain complementarity determining regions (LCDR) 1, 2 and 3, a heavy chain variable region (VH), or a light chain variable region (VL). Antibody fragments include a Fab fragment, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a $F(ab)_2$ 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 CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a domain antibody (dAb) fragment (Ward *et al* (1989) *Nature* 341:544- 546), which consists of a VH domain. VH and VL domains may 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 PCT Intl. Publ. Nos. WO1998/44001, WO1988/01649, WO1994/13804, and WO1992/01047. These antibody fragments are obtained using well known techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are full length antibodies.

The phrase "isolated antibody" refers to an antibody or antibody fragment that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody specifically binding CD38 is substantially free of antibodies that specifically bind antigens other than human CD38). An isolated antibody that specifically binds CD38, however, 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) provides a standardized numbering and definition of antigen-binding sites. The correspondence between CDRs, HVs and IMGT delineations is described in Lefranc *et al.*, Dev Comparat Immunol 27:55-77, 2003.

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

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

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

"Human-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 wherein 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, "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, for example 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 for example Fab arm exchange to generate 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 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. Epitope may be composed of contiguous and/or discontiguous amino acids that form a conformational spatial unit. For a discontiguous 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, substitutions, insertions or deletions.

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

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

"Treat" or "treatment" refers to therapeutic treatment wherein the object is to slow down (lessen) an undesired physiological change or disease, such as the development, expansion or spread of tumor or tumor cells, or to provide a beneficial or desired clinical outcome during treatment. 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" may 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 cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement dependent cytotoxicity (CDC), apoptosis, necrosis, inhibition of CD38 enzymatic activity, or by 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 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.

One embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody that 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 for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), 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) for a time sufficient to treat AML.

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

An exemplary 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) or minimally to residues KRN and VQLT (SEQ ID NO: 14) as shown above is daratumumab (see Intl. Pat. Publ. No. WO2006/0998647). Daratumumab comprises the VH and the 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/κ subtype. 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.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and

a light chain variable region (VL) of SEQ ID NOs: 4 and 5, respectively, for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising 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, for a time sufficient to treat AML.

SEQ ID NO: 1
MANCEFSPVSGDKPCCRLSRRAQLCLGVSILVLILVVVLAVVVPRWRQQWSGPGT
TKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCNITEEDYQPLM
KLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLEDTLLGYLADDLTWCGEFN
TSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSKIFDK
NSTFGSVEVHNLQPEKVQTLEAWVIHGGREDSRDLCQDPTIKELESIISKRNIQFSC
KNIYRPDKFLQCVKNPEDSSCTSEI

SEQ ID NO: 2
SKRNIQFSCKNIYR

SEQ ID NO: 3
EKVQTLEAWVIHGG

SEQ ID NO: 4
EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSA
ISGSGGGTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCAKDK
ILWFGEPVFDYWGQGTLVTVSS

SEQ ID NO: 5
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYD
ASNRATGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQ
GTKVEIK

SEQ ID NO: 6
SFAMS

SEQ ID NO: 7
AISGSGGGTYYADSVKG

SEQ ID NO: 8
DKILWFGEPVFDY

SEQ ID NO: 9
RASQSVSSYLA

SEQ ID NO: 10
DASNRAT

SEQ ID NO: 11
QQRSNWPPTF

SEQ ID NO: 12
EVQLLESGGGLVQPGGSLRLSCAVSGFTFNSFAMSWVRQAPGKGLEWVSAISGSG
GGTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYFCAKDKILWFGEPVF
DYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN
SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKRV
EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE
VKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVS
NKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN
HYTQKSLSLSPGK

SEQ ID NO: 13
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAT
GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIKRTVAAP
SVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDS
KDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 14

VQLT

Antibodies may be evaluated for their competition with daratumumab having VH of SEQ ID NO: 4 and 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 unlabelled 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 antibody 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 20%, 30%, 40%, 50%, 60%, 70%, 80%, 85%, 90% , 95% or 100%. The epitope of the test antibody may further be defined for example by peptide mapping or hydrogen/deuterium protection assays using known methods, or by crystal structure determination.

Antibodies binding to the same region on CD38 as daratumumab 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 using well known *in vitro* methods and as described herein.

Other exempary anti-CD38 antibodies that may be used in any embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, are:

mAb003 comprising the VH and VL sequences of SEQ ID NOs: 15 and 16, 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: 15

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGRVIPF
LGIANSAQKFQGRVTITADKSTSTAY
MDLSSLRSEDTAVYYCARDDIAALGPFDYWGQGTLVTVSSAS

SEQ ID NO: 16

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPRTFGQGTKVEIK;

mAb024 comprising the VH and VL sequences of SEQ ID NOs: 17 and 18, 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: 17

EVQLVQSGAEVKKPGESLKISCKGSGYSFSNYWIGWVRQMPGKGLEWMGIIYPH
DSDARYSPSFQGQVTFSADKSISTAYLQWSSLKASDTAMYYCARHVGWGSRYW
YFDLWGRGTLVTVSS

SEQ ID NO: 18

EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPRLLIYDASNRAT
GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK;

MOR-202 (MOR-03087) comprising the VH and VL sequences of SEQ ID NOs: 19 and 20, 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: 19

QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYMNWVRQAPGKGLEWVSGISGD
PSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDLPLVYTGFA
YWGQGTLVTVSS

SEQ ID NO: 20

DIELTQPPSVSVAPGQTARISCSGDNLRHYYVYWYQQKPGQAPVLVIYGDSKRPS
GIPERFSGSNSGNTATLTISGTQAEDEADYYCQTYTGGASLVFGGGTKLTVLGQ;

Isatuximab; comprising the VH and VL sequences of SEQ ID NOs: 21 and 22, 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 21:
QVQLVQSGAEVAKPGTSVKLSCKASGYTFTDYWMQWVKQRPGQGLEWIGT
IYPGDGDTGYAQKFQGKATLTADKSSKTVYMHLSSLASEDSAVYYCARGD
YYGSNSLDYWGQGTSVTVSS

SEQ ID NO: 22:
DIVMTQSHLSMSTSLGDPVSITCKASQDVSTVVAWYQQKPGQSPRRLIYS
ASYRYIGVPDRFTGSGAGTDFTFTISSVQAEDLAVYYCQQHYSPPYTFGG
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, Intl. Pat. Publ. No. WO06/125640, Intl. Pat. Publ. No. WO07/042309, Intl. Pat. Publ. No. WO08/047242 or Intl. Pat. Publ. No. WO14/178820.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) of SEQ ID NOs: 15 and 16, respectively, for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) of SEQ ID NOs: 17 and 18, respectively, for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) of SEQ ID NOs: 19 and 20, respectively, for a time sufficient to treat AML.

Another embodiment of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, is a method of treating a subject having acute myeloid leukemia (AML), comprising administering to the subject in need thereof an anti-CD38 antibody comprising a heavy chain variable region (VH) and a light chain variable region (VL) of SEQ ID NOs: 21 and 22, respectively, for a time sufficient to treat AML.

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 function 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, for example 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 induces killing of AML cells that express CD38 by apoptosis.

The anti-CD38 antibodies used in the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may induce killing of AML cells by apoptosis. Methods for evaluating apoptosis are well known, and include for example annexin IV staining using standard methods. The anti-CD38 antibodies used in the methods of the invention may induce apoptosis in about 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% of cells.

In the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 induces killing of AML cells that express CD38 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 induces killing of AML cells that express CD38 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 AML cells that express CD38 by ADCP.

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 AML cells that express CD38 by ADCC and CDC.

"Antibody-dependent cellular cytotoxicity", "antibody-dependent cell-mediated cytotoxicity" or "ADCC" is a mechanism for inducing cell death that depends upon the interaction of antibody-coated target cells with effector cells possessing lytic activity, such as natural killer cells, monocytes, macrophages and neutrophils via Fc gamma receptors (FcγR) expressed on effector cells. For example, NK cells express FcγRIIIa, whereas monocytes express FcγRI, FcγRII and FcvRIIIa. 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, 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. Exemplary effector cells for such assays include peripheral blood mononuclear cells (PBMC) and NK cells. Exemplary target cells include Daudi cells (ATCC® CCL-213™) or B cell leukemia or lymphoma tumor cells expressing CD38. In an exemplary assay, target cells are labeled with 20 μCi of ^{51}Cr for 2 hours and washed extensively. Cell concentration of the target cells may be adjusted to 1×10^{6} cells/ml, and anti-CD38 antibodies at various concentrations are added. Assays are started by adding Daudi cells at an effector:target cell ratio of 40:1. After incubation for 3 hr at 37°C assays are stopped by centrifugation, and ^{51}Cr release from lysed cells are measured in a scintillation counter. Percentage of cellular cytotoxicity may be calculated as % maximal lysis which may be induced by adding 3% perchloric acid to target cells. 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 (cell lysis induced by 3% perchloric acid).

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

"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. CDC of CD38-expressing cells may be measured for example by plating Daudi cells at 1×10^{5} cells/well (50 µl/well) in RPMI-B (RPMI supplemented with 1% BSA), adding 50 µl anti-CD38 antibodies to the wells at final concentration between 0-100 µg/ml, incubating the reaction for 15 min at room temperature, adding 11 µl of pooled human serum to the wells, and incubating the reaction for 45 min at 37° C. Percentage (%) lysed cells may be detected as % propidium iodide stained cells in FACS assay using standard methods. 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 FcγRIIIa binding without altering antigen binding or CDC activity. Such mAbs may be achieved using different methods reported to lead to the

successful expression of relatively high defucosylated antibodies bearing the biantennary complex-type of Fc oligosaccharides such as control of culture osmolality (Konno *et al.*, Cytotechnology 64:249-65, 2012), application of a variant CHO line Lec13 as the host cell line (Shields *et al.*, J Biol Chem 277:26733-26740, 2002), application of a variant CHO line EB66 as the host cell line (Olivier *et al.*, MAbs ;2(4), 2010; Epub ahead of print; PMID:20562582), application of a rat hybridoma cell line YB2/0 as the host cell line (Shinkawa *et al.*, J Biol Chem 278:3466-3473, 2003), introduction of small interfering RNA specifically against the α 1,6-fucosyltrasferase (*FUT8)* gene (Mori *et al.*, Biotechnol Bioeng88:901-908, 2004), or coexpression 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 Chem281:5032-5036, 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.

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 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 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" means the amount of the fucose monosaccharide within the sugar chain at Asn297. The relative amount of fucose is the percentage of fucose-containing structures related to all glycostructures. These may be characterized and quantified by multiple methods, for example: 1) using MALDI-TOF of N-glycosidase F treated sample (e.g. complex, hybrid and oligo- and high-mannose structures) as described in Intl. 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 can be labeled with a fluorophore, separated and identified by various complementary techniques which allow: fine characterization of the glycan structures by matrix-assisted laser desorption ionization (MALDI) mass spectrometry by comparison of the experimental masses with the theoretical masses, determination of the degree of sialylation by ion exchange HPLC (GlycoSep C), separation and quantification of the oligosacharride 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%.

The anti-CD38 antibodies used in the methods described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may induce killing of AML cells by modulation of CD38 enzymatic activity. CD38 is a multifunctional ectoenzme with ADP-ribosyl cyclase activity catalyzing the formation of cyclic ADP-ribose (cADPR) and ADPR from NAD^+. CD38 also catalyzes the exchange of the nicotinamide group of $NADP^+$ with nicotinic acid under acidic conditions, to yield $NAADP^+$ (nicotinic acid-adenine dinucleotide phosphate). Modulation of the enzymatic activity of human CD38 with anti-CD38 antibodies used in the methods of the invention

may be measured in an assay described in Graeff *et al.*, J. Biol. Chem. 269, 30260-30267 (1994). For example, substrate NGD^+ may be incubated with CD38, and the modulation of the production of cyclic GDP-ribose (cGDPR) may be monitored spectrophotometrically at excitation at 340 nM and emission at 410 nM at different time points after addition of the antibody at various concentrations. Inhibition of the synthesis of cADPR can be determined according to the HPLC method described in Munshi *et al.*, J. Biol. Chem. 275, 21566-21571 (2000). The anti-CD38 antibodies used in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may inhibit CD38 enzymatic activity by at least about 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95% or 100% .

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 a heavy chain of SEQ ID NO: 12 and a 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 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. "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 may be used as a query sequence to perform a search against public or patent databases to, for example, identify related sequences. Exemplary programs used to perform such searches are the XBLAST or BLASTP programs (http_//www_ncbi_nlm/nih_gov), or the GenomeQuest™ (GenomeQuest, Westborough, MA) suite using the default settings. Exemplary substitutions that may be made to the anti-CD38 antibodies used in the methods of the invention are for example conservative substitutions with an amino acid having similar charge, hydrophobic, or stereochemical characteristics. Conservative substitutions may also be made to improve antibody properties, for example stability or affinity, or to improve antibody effector functions. 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, or 15 amino acid substitutions may be made for example to the heavy or the light chain of the anti-CD38 antibody. Furthermore, any native residue in the heavy or light chain may also be substituted with alanine, as has been previously described for alanine scanning mutagenesis (MacLennan *et al.*, Acta Physiol Scand Suppl 643:55-67, 1998; Sasaki *et al.*, Adv Biophys 35:1-24, 1998). Desired amino acid substitutions may be determined by those skilled in the art at the time such substitutions are desired. Amino acid substitutions may be done for example by PCR mutagenesis (U.S. Pat. No. 4,683,195). Libraries of variants may be generated using well known methods, for example using random (NNK) or non-random codons, for example DVK codons, which encode 11 amino acids (Ala, Cys, Asp, Glu, Gly, Lys, Asn, Arg, Ser, Tyr, Trp) and screening the libraries for variants with desired properties. The generated variants may be tested for their binding to CD38 and their ability to induce apoptosis or modulate CD38 enzymatic activity using methods described herein.

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 a K_D equal to or less than about $1x10^{-8}$ M, for example $5x10^{-9}$ M, $1x10^{-9}$ M, $5x10^{-10}$ M, $1x10^{-10}$ M, $5x10^{-11}$ M, $1x10^{-11}$ M, $5x10^{-12}$ M, $1x10^{-12}$ M, $5x10^{-13}$ M, $1x10^{-13}$ M, $5x10^{-14}$ M, $1x10^{-14}$ M or $5x10^{-15}$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 $1x10^{-8}$ M. Another exemplary affinity is equal to or less than $1x10^{-9}$ M.

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

For example, bispecific antibodies of the invention may be generated *in vitro* in a cell-free environment by introducing asymmetrical mutations in the CH3 regions of two monospecific homodimeric antibodies and forming the bispecific heterodimeric antibody from two parent monospecific homodimeric antibodies in reducing conditions to allow disulfide bond isomerization according to methods described in Intl.Pat. Publ. No. WO2011/131746. In the methods, the first monospecific bivalent antibody (e.g., anti-CD38 antibody) and the second monospecific bivalent antibody are engineered to have certain substitutions at the CH3 domain that promote heterodimer stability; the antibodies are incubated together under reducing conditions sufficient to allow the cysteines in the hinge region to undergo disulfide bond isomerization; thereby generating the bispecific antibody by Fab arm exchange. The incubation conditions may optimally be restored to non-reducing. Exemplary reducing agents that may be used are 2- mercaptoethylamine (2-MEA), dithiothreitol (DTT), dithioerythritol (DTE), glutathione, tris(2-carboxyethyl)phosphine (TCEP), L-cysteine and beta-mercaptoethanol, preferably a reducing agent selected from the group consisting of: 2- mercaptoethylamine,

dithiothreitol and tris(2-carboxyethyl)phosphine. For example, incubation for at least 90 min at a temperature of at least 20°C in the presence of at least 25 mM 2-MEA or in the presence of at least 0.5 mM dithiothreitol at a pH of from 5-8, for example at pH of 7.0 or at pH of 7.4 may be used.

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

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

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is conjugated to a toxin. Conjugation methods and suitable toxins are well known.

AML diagnosis is performed by a physician according to guidelines available, for example according to the World Health Organization (WHO) classification of AML (Brunning et al., World Health Organization Classificaiton of Tumors, 3, pp77-80; eds. Jaffe et al., Pathology and Genetics of Tumours of Haematopoietic and Lymphoid Tissues) and according to guidelines available for example at National Comprehensive Cancer Network (http://_www_nccn.org/_professionals/_physician_gls/_f_guidelines_asp#site). The WHO classification incorporates clinical features, cytogenetics, immunophenotype, morphology and genetics in order to define biologically homogenous subgroups having therapeutic and prognostic relevance, and divides AML to four main subtypes: AML with recurrent genetic abnormalities, AML with multilineage dysplasia, therapy-related AML, and not otherwise categorized AML.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is AML with at least one genetic abnormality.

AML may be associated with a translocation between chromosomes 8 and 21, translocation or inversion in chromosome 16, translocation between chromosomes 15 and 17, or changes in chromosome 11.

Common chromosomal rearrangements associated with AML are translocations t(8; 21)(q22; q22) (AML1/ETO), inv(16)(p13; q22) or t(16; 16)(p13; q22); (CBFβ/MYH11) or t(15; 17)(q22; q12); (PML/RARA). Patients with these favorable chromosomal translocations may be more susceptible to treatment and achieve higher complete remission (CR) rates.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with a translocation between chromosomes 8 and 21, translocation or inversion in chromosome 16, translocation between chromosomes 15 and 17, or changes in chromosome 11.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with a chromosomal abnormality t(8; 21)(q22; q22) (AML1/ETO), inv(16)(p13; q22) or t(16; 16)(p13; q22); (CBFβ/MYH11) or t(15; 17)(q22; q12); (PML/RARA).

Somatic mutations in various genes have been identified as being relevant to AML pathogenesis. These include mutations in fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), isocitrate dehydrogenase 1(IDH1), isocitrate dehydrogenase 2 (IDH2), DNA (cytosine-5)-methyltransferase 3 (DNMT3A), CCAAT/enhancer binding protein alpha (CEBPA), U2 small nuclear RNA auxiliary factor 1(U2AF1), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), structural maintenance of chromosomes 1A (SMC1A) and structural maintenance of chromosomes 3 (SMC3) (The Cancer Genome Atlas Research Network; N Engl J Med 368:2059-74, 2013).

Activating mutations in the FLT3 gene have been described in approximately 20-30% of newly diagnosed AML patients. These include FLT3-ITD, internal tandem duplication mutations as a result of duplication and tandem insertion of parts of the juxtamembrane domain of the FLT3 gene (Schnittger *et al.*, Blood 100:59-66, 2002) and D835 mutations in the FLT3 kinase domain. Patients with FLT3-ITD mutations appear to have reduced overall survival (OS) with increased relapse rate (Kottaridis *et al.*, Blood 98: 1752-9, 2001; Yanada *et al.*, Leukemia 19: 1345-9, 2005).

Mutations in IDH1 and IDH2 are present in about 15% of newly diagnosed patients. IDH1 mutations include substitutions R132H, R132X (X being any amino acid) and R100Q/R104V/F108L/R119Q/I130V and IDH2 mutations include substitutions R140Q and R172. IDH1/2 mutations are associated with poorer prognosis, except that $IDH2^{R140Q}$ is associated with somewhat prolonged survival (Molenaar *et al.*, Biochim Biophys Acta 1846: 326-41, 2014). IDH1/2 mutation frequency increases with disease progression (Molenaar et al., Biochim Biophys Acta 1846: 326-41, 2014).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with one or more mutations in a fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), isocitrate dehydrogenase 1(IDH1), isocitrate dehydrogenase 2 (IDH2), DNA (cytosine-5)-methyltransferase 3 (DNMT3A), CCAAT/enhancer binding protein alpha (CEBPA), U2 small nuclear RNA auxiliary factor 1(U2AF1), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), structural maintenance of chromosomes 1A (SMC1A) and structural maintenance of chromosomes 3 (SMC3).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with one or more mutations in fms-related tyrosine kinase 3 (FLT3).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with FLT3-ITD.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with one or more mutations in isocitrate dehydrogenase 1(IDH1) or isocitrate dehydrogenase 2 (IDH2).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with mutations R132H, R132X or R100Q/R104V/F108L/R119Q/I130V in isocitrate dehydrogenase 1 (IDH1).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is associated with mutations R140Q and R172 in isocitrate dehydrogenase 2 (IDH2).

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is AML with multilineage dysplasia.

AML associated with multilineage dysplasia is characterized by dysplasia in two or more myeloid cell lineage, and by at least 20% increased blasts in either the blood or bone marrow.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is therapy-related AML.

Therapy-related AML is a result of prior chemotherapy and/or radiation therapy, and may occur several years after exposure to the mutagenic agent. More than 90% of

patients with therapy-related AML exhibit chromosomal abnormalities, including those of chromosomes 5 and/or 7.

Chromosomal rearrangements may be identified using well-known methods, for example fluorescent in situ hybridization, karyotyping, Southern blot, or sequencing.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is undifferentiated AML (M0), AML with minimal maturation (M1), AML with maturation (M2), acute myelomonocytic leukemia (M4), acute monocytic leukemia (M5), acute erythroid leukemia (M6), acute megakaryoblastic leukemia (M7), acute basophilic leukemia, acute panmyelosis with fibrosis or myeloid sarcoma.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is in remission.

AML in remission is typically defined as normocellular marrow with less than 5% blasts, normal peripheral blood count with >100,000/mm^3 platelets and >1,000/mm^3 neutrophils.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is relapsed or refractory.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the patient having AML has been treated with idarubicin, cytrabine or hydroxyurea.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is adult AML.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, AML is pediatric AML.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is administered as a remission induction, post-remission or maintenance therapy.

Various qualitative and/or quantitative methods may be used to determine if a subject has relapsed, is resistant, has developed or is susceptible to developing a resistance to treatment with a drug or a therapeutic. Symptoms that may be associated with relapse and/or resistance include, for example, a decline or plateau of the well-being of the patient, an increase in the size of a tumor or tumor burden, 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 relapsed or has developed or is susceptible to developing resistance to a drug or a therapeutic. The symptoms associated with cancer may vary according to the type of cancer. For example, symptoms associated with AML may include weakness, tiredness, feeling dizzy or cold, headaches, frequent nosebleeds, excess bruising or bleeding gums.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is administered in combination with at least one additional therapeutic.

AML may be treated with cytarabine (cytosine arabinoside, or ara-C) and/or antharcycline drugs such as doxorubicin, daunorubicin, daunomycin, idarubicin and mitoxantrone. Other chemotherapeutic drugs that may be used to treat AML include Hydroxyurea (Hydrea®), Decitabine (Dacogen®), Cladribine (Leustatin®, 2-CdA), Fludarabine (Fludara®), Topotecan, Etoposide (VP-16), 6-thioguanine (6-TG), Corticosteroid drugs, such as prednisone or dexamethasone (Decadron®), methotrexate (MTX), 6-mercaptopurine (6-MP) or Azacitidine (Vidaza®).

Other drugs that may be used to treat AML are all-trans-retinoic acid (ATRA), tretinoin, or Vesanoid® and arsenic trioxide (ATO, Trisenox®). ATRA and arsenic trioxide may be used to treat acute promyelocytic leukemia.

In some embodiments, the anti-CD38 antibody is administered to a patient in combination with cytarabine, daunorubicin/daunomycin, idarubicin, mitoxantrone, hydroxyurea, decitabine, cladribine, fludarabine, topotecan, etoposide 6-thioguanine, corticosteroid, prednisone, dexamethasone, methotrexate, 6-mercaptopurine or azacitidine.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is administered to a patient in combination with decitabine.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody is administered to a patient in combination with cytarabine and doxorubicin.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject has received or will receive radiotherapy.

Radiotherapy may be external beam radiation, intensity modulated radiation therapy (IMRT), focused radiation, or any form of radiosurgery including Gamma Knife, Cyberknife, Linac, and interstitial radiation (e.g. implanted radioactive seeds, GliaSite balloon), and/or with surgery.

Focused radiation methods that may be used include stereotactic radiosurgery, fractionated stereotactic radiosurgery, and intensity-modulated radiation therapy (IMRT). It is apparent that stereotactic radiosurgery involves the precise delivery of radiation to a tumorous tissue, for example, a brain tumor, while avoiding the surrounding non-tumorous, normal tissue. The dosage of radiation applied using stereotactic radiosurgery may vary, typically from 1 Gy to about 30 Gy, and may encompass intermediate ranges including, for example, from 1 to 5, 10, 15, 20, 25, up to 30 Gy in dose. Because of noninvasive fixation devices, stereotactic radiation need not be delivered in a single treatment. The treatment plan may be reliably duplicated day-to-day, thereby allowing multiple fractionated doses of radiation to be delivered. When used to treat a tumor over time, the radiosurgery is referred to as "fractionated stereotactic radiosurgery" or FSR. In contrast, stereotactic radiosurgery refers to a one-session treatment. Fractionated stereotactic radiosurgery may result in a high therapeutic ratio, i.e., a high rate of killing of tumor cells and a low effect on normal tissue. The tumor and the normal tissue respond differently to high single doses of radiation vs. multiple smaller doses of radiation. Single large doses of radiation may kill more normal tissue than several smaller doses of radiation may. Accordingly, multiple smaller doses of radiation can kill more tumor cells while sparing normal tissue. The dosage of radiation applied using fractionated stereotactic radiation may vary from range from 1 Gy to about 50 Gy, and may encompass intermediate ranges including, for example, from 1 to 5, 10, 15, 20, 25, 30, 40, up to 50 Gy in hypofractionated doses. Intensity-modulated radiation therapy (IMRT) may also be used. IMRT is an advanced mode of high-precision three-dimensional conformal radiation therapy (3DCRT), which uses computer-controlled linear accelerators to deliver precise radiation doses to a malignant tumor or specific areas within the tumor. In 3DCRT, the profile of each radiation beam is shaped to fit the profile of the target from a beam's eye view (BEV) using a multileaf collimator (MLC), thereby producing a number of beams. IMRT allows the radiation dose to conform more precisely to the three-dimensional (3-D) shape of the tumor by modulating the intensity of the radiation beam in multiple small volumes. Accordingly, IMRT allows higher radiation doses to be focused to regions within the tumor while minimizing the dose to surrounding normal critical structures. IMRT improves the ability to conform the treatment volume to concave tumor shapes, for example, when the tumor is wrapped around a vulnerable structure, such as the spinal cord or a major organ or blood vessel.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject is undergoing hematopoietic stem cell transplantation (HSCT).

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the HSCT is allogeneic, autologous or synegeneic, i.e. the donor is a twin. Autologous HSCT comprises the extraction of HSC from the subject and freezing of the harvested HSC. After myeloablation, the subject's stored HSC are transplanted into the subject. Allogeneic HSCT involves HSC obtained from an allogeneic HSC donor who has an HLA type that matches the subject.

"Hematopoietic stem cell transplantation" is the transplantation of blood stem cells derived from the bone marrow (in this case known as bone marrow transplantation), blood (such as peripheral blood and umbilical cord blood), or amniotic fluid.

"Undergoing hematopoietic stem cell transplantation" means that the patient did already receive, is receiving or will receive HSCT.

In some embodiments of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the patient has completed chemotherapy and/or radiation therapy prior to HSCT.

Patients may be treated with chemotherapy and/or radiation therapy prior to HSCT (so-called pre-transplant preparation) to eradicate some or all of the patient's hematopoietic cells prior to transplant. The patient may also be treated with immunosuppressants in case of allogeneic HSCT. An exemplary pre-transplant preparation therapy is high-dose melphalan (see for example Skinner *et al.,* Ann Intern Med 140:85-93, 2004; Gertz *et al.,* Bone Marrow Transplant 34: 1025-31, 2004; Perfetti *et al.,* Haematologica 91:1635-43, 2006). The radiation therapy that may be employed in pre-transplant treatment may be carried out according to commonly known protocols in this field. Radiation therapy may also be provided simultaneously, sequentially or separately with the anti-CD38 antibody.

In some embodiments described herein, and in some embodiments of each and every one of the numbered embodiments listed below, the subject having AML 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 AML, comprising administering to a patient 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), 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 an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with dacogen, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3)

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with a second therapeutic agent, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with dacogen, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with doxorubicin, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for use in treating a subject having AML in combination with cytrabine and doxorubicin, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 15 and the VL of SEQ ID NO: 16 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 17 and the VL of SEQ ID NO: 18 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 19 and the VL of SEQ ID NO: 20 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a mutation in fms-related tyrosine kinase 3 (FLT3).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a FLT3-ITD mutation.

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a R140Q mutation in isocitrate dehydrogenase 2 (IDH2).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

The invention also provides an anti-CD38 antibody comprising the VH of SEQ ID NO: 21 and the VL of SEQ ID NO: 22 for use in treating a subject having AML, wherein the subject has a R882H mutation in DNA (cytosine-5)-methyltransferase 3 (DNMT3A).

Administration/ Pharmaceutical Compositions

In the methods of the invention described herein, 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 can be used. These solutions are sterile and generally free of particulate matter. They may be sterilized by conventional, well-known sterilization techniques (*e.g.*, filtration). The compositions may contain pharmaceutically acceptable auxiliary substances as required to approximate physiological conditions such

as pH adjusting and buffering agents, stabilizing, thickening, lubricating and coloring agents, etc. The concentration of the molecules or antibodies of the invention in such pharmaceutical formulation may vary widely, *i.e.*, from less than about 0.5%, usually to at least about 1% to as much as 15 or 20% by weight and will be selected primarily based on required dose, fluid volumes, viscosities, etc., according to the particular mode of administration selected. Suitable vehicles and formulations, inclusive of other human proteins, e.g., human serum albumin, are described, for example, in e.g. Remington: The Science and Practice of Pharmacy, 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 described herein, and in some embodiments of each and every one of the numbered embodiments listed below, 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 described herein, 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 AML is sufficient to alleviate or at least partially arrest the disease being treated ("therapeutically effective amount") and may be sometimes 0.005 mg to about 100 mg/kg, e.g. about 0.05 mg to about 30 mg/kg or about 5 mg to about 25 mg/kg, or about 4 mg/kg, about 8 mg/kg, about 16 mg/kg or about 24 mg/kg , or for example about 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 mg/kg, but may even higher, for example about 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 30, 40, 50, 60, 70, 80, 90 or 100 mg/kg.

A fixed unit dose may also be given, for example, 50, 100, 200, 500 or 1000 mg, or the dose may be based on the patient's surface area, e.g., 500, 400, 300, 250, 200, or 100 mg/m^2. Usually between 1 and 8 doses, (e.g., 1, 2, 3, 4, 5, 6, 7 or 8) may be administered to treat AML, 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 described herein, 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 described herein, 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 described herein, 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 described herein, 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 described herein, 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 described herein, 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/m^2/day PO or 25 mg/m^2/day PO.

The anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be administered in combination with dacogen.

Dacogen may be administered for a minimum of 4 cycles repeated every 6 weeks at 15 mg/m^2 *i.v.* over 3 hours repeated every 8 hours for 3 days. Alternatively, dacogen may be administered 20 mg/m^2 *i.v.* over 1 hour repeated daily for 5 days, and the cycle repeated every 4 weeks.

The anti-CD38 antibody in the methods of the invention described herein, and in some embodiments of each and every one of the numbered embodiments listed below, may be administered in combination with cytrabine and doxorubicin.

Cytarabine may be administered 2 to 3 g/m^2 *i.v.* over 1-3 hours every twelve hours for up to 12 doses.

Doxorubicin may be administered 40 to 60 mg/m^2 *i.v.* every 21 to 28 days, or 60 to 75 mg/m^2 *i.v.* once every 21 days.

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

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 acute myeloid leukemia (AML).

2. An anti-CD38 antibody for use in treating a subject having AML, in combination with a second therapeutic agent, wherein the second therapeutic agent
    a. is optionally cytarabine, daunorubicin, idarubicin, mitoxantrone, hydroxyurea, decitabine, cladribine, fludarabine, topotecan, etoposide 6-thioguanine, corticosteroid, prednisone, dexamethasone, methotrexate, 6-mercaptopurine, azacitidine, arsenic trioxide or all-trans retinoic acid; and/or
    b. increases surface expression of CD38.
3. A combination of an anti-CD38 antibody and all-trans retinoic acid for use in treating a subject having AML.
4. A combination of an anti-CD38 antibody and decitabine for use in treating a subject having AML.
5. A combination of an anti-CD38 antibody and cytarabine and/or doxorubicin for use in treating a subject having AML.
6. The anti-CD38 antibody for use according to embodiment 1 or 2, or the combination according to embodiment 3-5, 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.
7. The anti-CD38 antibody for use according to embodiment 1, 2 or 6, or the combination according to embodiment 3-6, wherein the anti-CD38 antibody induces killing of AML cells that express CD38 by apoptosis.
8. The anti-CD38 antibody for use according to embodiment 1, 2, 6 or 7 or the combination according to embodiment 3-7, wherein the anti-CD38 antibody binds to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1).
9. The anti-CD38 antibody for use according to embodiment 1, 2, 6-8, or the combination according to embodiment 3-8, wherein the anti-CD38 antibody:
    a. is of IgG1, IgG2, IgG3 or IgG4 isotype;
    b. 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%; or
    c. comprise a substitution in the antibody Fc at amino acid positions 256, 290, 298, 312, 356, 330, 333, 334, 360, 378 or 430, when residue numbering according to the EU index.

10. The anti-CD38 antibody for use according to embodiment 1, 2, 6-9, or the combination according to embodiment 3-9, wherein the anti-CD38 antibody comprises

a. the heavy chain complementarity determining regions (HCDR) 1 (HCDR1), 2 (HCDR2) and 3 (HCDR3) sequences of SEQ ID NOs: 6, 7 and 8, respectively;

b. the light chain complementarity determining regions (LCDR) 1 (LCDR1), 2 (LCDR2) and 3 (LCDR3) sequences of SEQ ID NOs: 9, 10 and 11, respectively;

c. HCDR1, HCDR2, HCDR3, LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NOs: 6, 7, 8, 9, 10 and 11, respectively;

d. the heavy chain variable region (VH) of SEQ ID NO: 4 and the light chain variable region (VL) of SEQ ID NO: 5;

e. 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; or

f. the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13.

11. The anti-CD38 antibody for use according to embodiment 1, 2, 6-10, or the combination according to embodiment 3-10, wherein AML with at least one genetic abnormality, AML with multilineage dysplasia, therapy-related AML, undifferentiated AML, AML with minimal maturation, AML with maturation, acute myelomonocytic leukemia, acute monocytic leukemia, acute erythroid leukemia, acute megakaryoblastic leukemia, acute basophilic leukemia, acute panmyelosis with fibrosis or myeloid sarcoma.

12. The anti-CD38 antibody for use according to embodiment 1, 2, 6-11, or the combination according to embodiment 3-11, wherein the anti-CD38 antibody is administered as a remission induction, post-remission or maintenance therapy.

13. The anti-CD38 antibody for use according to embodiment 1, 2, 6-12, or the combination according to embodiment 3-12, wherein the at least one genetic abnormality is a translocation between chromosomes 8 and 21, a translocation or an inversion in chromosome 16, a translocation between chromosomes 15 and 17, changes in chromosome 11, or mutation in fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), isocitrate dehydrogenase 1(IDH1), isocitrate dehydrogenase 2 (IDH2), DNA (cytosine-5)-methyltransferase 3 (DNMT3A),

CCAAT/enhancer binding protein alpha (CEBPA), U2 small nuclear RNA auxiliary factor 1(U2AF1), enhancer of zeste 2 polycomb repressive complex 2 subunit (EZH2), structural maintenance of chromosomes 1A (SMC1A) or structural maintenance of chromosomes 3 (SMC3).

14. The anti-CD38 antibody for use according to embodiment 1, 2, 6-13, or the combination according to embodiment 3-13, wherein the at least one genetic abnormality is a translocation t(8; 21)(q22; q22), an inversion inv(16)(p13; q22), a translocation t(16; 16)(p13; q22), a translocation t(15; 17)(q22; q12), a mutation FLT3-ITD, mutations R132H or R100Q/R104V/F108L/R119Q/I130V in IDH1 or mutations R140Q or R172 in IDH2.

15. The anti-CD38 antibody for use according to embodiment 1, 2, 6-14, or the combination according to embodiment 3-14, wherein the anti-CD38 antibody and the at least one therapeutic agent are administered simultaneously, sequentially or separately.

16. The anti-CD38 antibody for use according to embodiment 1, 2, 6-15, or the combination according to embodiment 3-15, wherein

a. the subject is further treated or has been treated with radiotherapy; or
b. the subject has received hematopoietic stem cell transplantation.

Examples

Example 1. Efficacy of daratumumab in AML cell lines

Several AML cell lines were used to evaluate surface expression of CD38 and possible efficacy of daratumumab in inducing AML cell killing. Expression of complement inhibitory proteins (CIP) CD46, CD55 and CD59 in the AML cell lines was assessed to evaluate possible correlation between expression of CIP and CDC.

Methods:

ADCC

In vitro ADCC assays were performed using AML tumor cell lines and Peripheral Blood Mononuclear Cells (PBMC) as effector cells at a ratio of 50:1. One hundred µl of target (tumor) cells ($1x10^4$ cells) were added to wells of 96-well U-bottom plates. An additional 100 µl was added with or without antibody, and the plates were incubated for 30 minutes at room temperature (RT) before adding effector cells (PBMC). Seventy five µl of PBMCs at concentration $6.66x10^6$ cells/ml was added to the wells of the plates, and the

plates were incubated at 37°C for 6 hours. Plates were centrifuged at 250 g for 4 minutes, 50 µl of supernatant removed per well and cell lysis was measured using the CellTiter-Glo® assay (Promega).

CDC

Target cells were harvested and adjusted to a concentration of $80x10^4$cells/ml. Twelve µl of target cells were added to wells of a 96-well plate, and serial dilution of antibodies added onto the cells. The wells were incubated for 15 minutes, after which human serum high in complement was added at a final concentration of 10%. Reaction mixture was incubated for 21/2 hours at 37°C, and cell lysis was measured using the CellTiter-Glo® assay (Promega).

Apoptosis

One ml of target cells (5×10^5 cells/ml) were added to the well of a 24-well plate, together with test antibody (1 µg/ml) in the presence or absence of rabbit anti-huIgG (10 µg/ml; F(ab')2 Fcγ-specific). Cells were incubated for 22 hours (5% CO2, 37°C). Thereafter, cells were harvested (1000 rpm, 5 min) and washed twice in PBS (1000 rpm, 5 min). Cells were resuspended in 250 µl binding buffer (Annexin-V Apoptosis kit, BD Biosciences) according to manufacturer's instruction, followed by flow cytometry analysis.

Apoptosis was measured by both early and late apoptosis (Q2 and Q3 in Figure 1A and Figure 1B).

CD38, CD46, CD55 and CD59 surface expression

Expression of receptors was analyzed by flow cytometry. The CD38 receptor number per cell was estimated using MESF kit using PE-labeled anti-CD38 antibody (R&D Systems). The receptor numbers were calculated as follows: Specific MESF/ABC = MESF/ABC (Test Antibody) – MESF/ABC (Isotype control antibody).

CD46, CD55 and CD59, surface expression was detected using FITC anti-human CD46, PE-anti-human CD55 and PE-anti-human CD59 antibodies (Beckton Dickinson) expressed as median fluorescent intensity (MFI).

Results

Table 1 shows the results of the experiments. **Figure 1** shows representative flow cytometry results of daratumumab-induced apoptosis in NB-4 cell line without **(Figure 1A)** or with **(Figure 1B)** crosslinking antibody. In this cell line, daratumumab induced apoptosis to a similar degree independent of the presence of the crosslinking agent (19.2% vs 18.3%).

In the AML cell lines, daratumumab did not induce significant ADCC or CDC; instead; daratumumab induced AML cell killing by apoptosis. In addition, no direct correlation was observed between CD38 expression and the extent of ADCC and CDC. The levels of complement inhibitory proteins (CIP) (CD46, CD55 and CD59) were evaluated to determine if these proteins affected CDC in response to daratumumab but no direct correlation was observed between CDC and CIP expression.

Table 1.

Cell line	CD38 #/cell	CD46 MFI	CD55 MFI	CD59 MFI	Apoptosis	CDC	ADCC
HL-60	64.50	ND	ND	ND	ND	ND	ND
Kasumi-1	120.2	ND	ND	ND	ND	ND	ND
ML-2	1,253.27	21.53	195.2	0.98	5%	0%	6.30%
MOLM-13	5,634.29	35.53	173.2	9.45	10-15%	0%	9.40%
MOLM-16	52,461.11	42.18	886.4	350.42	20-30%	5%	18.20%
MV-4-11	5,700.05	207.17	395.42	43.94	10-12%	0%	2.30%
NB4	9,370.73	58.25	345.4	66.2	18%	4%	18.30%
THP-1	39,488.19	58.7	375	27.1	5-7%	5%	11.30%

ND: not done
MFI: mean fluorescence intensity

Example 2. ATRA induces CD38 expression on AML cells

Effect of ATRA on CD38 surface expression was assessed in NB-4 AML cell line. Tumor cells were incubated at 37°C for 24 hours in the presence or absence of 10 nM or 100 nM ATRA. After 24 hour incubation, the cells were harvested and stained for CD38. ATRA induced ~10-fold increase in CD38 receptors in the NB-4 cell line. CD38 surface expression was assessed using FACS using PE-labeled anti-CD38 antibody (R&D Systems) (Table 2).

Table 2.

Treatment	PE-CD38 molecules/cell
DMSO	17238
10 nM ATRA	185737
100 nM ATRA	210570

Example 3. Efficacy of daratumumab in patient-derived xenograft (PDX) models

Methods

Patient tumor models AML 3406, AML 7577 and AML 8096 were used in the study.

AML3406 model: Patient tumor cells were positive for FLT-3ITD. Patient has a history of polycythemia versa, and received idarubicin/ cytrabine for induction chemotherapy. Patient also received Hudrea® (hydroxyurea).

AML 7577 model: Leukemic cells were collected from a 69-year old male with AML (FAB subtype M5). Patient had normal karyotype and following mutations: IDH2(R140Q); FLT3-ITD; DNMT3A R882H, NPM1, CEBPA insertion (SNP). Patient has a history of polycythemia versa, and received idarubicin/ cytrabine for induction chemotherapy. Patient also received Hudrea® (hydroxyurea).

AML 8096 model: Leukemic cells were collected from a 21-year old male with AML (FAB subtype M2). White blood cell count was 20X10e^9/L, from which 70% were blast cells. Patient had normal karyotype with wild type TP53, FLT3, NPM1, and insertion 570-587, 3GCACCC>4GCACCC in CEBPA exon1. Patient has a history of polycythemia versa, and received idarubicin/ cytrabine for induction chemotherapy. Patient also received Hudrea® (hydroxyurea).

5 million AML MNCs were T-cell depleted and transplanted via tail vein into 6-8 weeks old sub-lethaly irradiated NSG mice (n=10 per group). 4 to 6 weeks post-engraftment, bone marrow aspirates were collected from each mouse and were analyzed by flow cytometry to determine the level of leukemia engraftment (% of human $CD45^+$ $CD33^{+/-}$ cells). Based on engraftment levels, mice were randomized and conditioned with either IgG1 or daratumumab (DARA, pre-dosing at 0.5 mg/kg). 24 hours later, mice were untreated (Ctrl) or treated for 5 consecutive weeks with DARA or IgG1 alone (i.p, 10mg/kg once a week). 2-3 days after the last treatment, mice were sacrificed and bone marrow, spleen, peripheral blood and plasma were collected for analysis. Flow cytometry was performed to assess percentage of human $CD45^+CD33^+$ cells in the BM, SPL and PB of 3 AML patients engrafted in NSG mice (AML 3406 model: **Figure 2A;** AML 7577 model: **Figure 2B,** AML 8096 model: **Figure 2C**) and absolute number of the human $CD45^+CD33^+$ cells in bone marrow **(Figure 3A),** spleen **(Figure 3B)** and peripheral blood **(Figure 3C)** of one representative AML patient.

Results

Figure 2A, Figure 2B and Figure 2C show the efficacy of daratumumab in the AML 3406 model, AML 7577 model and the AML 8096 model, respectively, assessed by reduction in % leukemic $CD45^+CD33^+$ cells in bone marrow, spleen or peripheral blood. Daratumumab reduced tumor burden in spleen and peripheral blood in the AML 3406 model (Figure 2A), in peripheral blood in the AML 7577 model (Figure 2B), and in spleen in the AML 8096 model (Figure 2C).

Efficacy of daratumumab was also assessed by measuring daratumumab-induced reduction in total leukemic burden in bone marrow (Figure 3A), spleen (Figure 3B) and blood (Figure 3C) in the AML 3406 model. Daratumumab significantly reduced total leukemic burden in the AML 3406 model in spleen (Figure 3B) and in peripheral blood (Figure 3C).

Example 4. Effect of daratumumab on CD38 expression on AML blasts

Effect of daratumumab on CD38 expression on leukemic blasts was assessed in one representative AML model described in Example 3 after 5 weeks of treatment with daratumumab or isotype control using PE-labeled anti-CD38 antibody (R&D Systems).

Results

Figure 4A shows that treatment with daratumumab reduced expression of CD38 on leukemia blasts ($CD45^+CD33^+$ positive cells) in bone marrow, spleen and peripheral blood. **Figure 4B** shows that percentage of CD38-positive AML blasts were reduced after 5 weeks of treatment.

Example 5. Efficacy of daratumumab combination therapy in patient-derived xenograft (PDX) models

Efficacy of daratumumab in combination with dacogen or cytarabine and doxorubicin was assessed after 5 weeks of treatment.

5 million AML MNCs were T-cell depleted and transplanted via tail vein into 6-8 weeks old NSG mice (n=10 per group). 4 to 6 weeks post-engraftment, bone marrow aspirates were collected from each mouse and were analyzed by flow cytometry to determine the level of leukemia engraftment (% of human $CD45^+$ $CD33^{+/-}$ cells). Based on engraftment levels, mice were equally randomized and conditioned with either IgG1 or DARA (pre-dosing at 0.5 mg/kg). 24 hours later, mice were treated with IgG1 alone (i.p, 10mg/kg) once a week for five weeks, with DARA alone (i.p,10 mg/kg) once a week for five weeks, with decitabine alone (DAC) (0.5 mg/kg/day, i.p. for 3 consecutive days) for five weeks, with DAC + DARA (each week will consist of 3 consecutive days of DAC followed by DARA 2 days later), with a combination of cytarabine (i.v, 50 mg/kg) and doxorubicin (i.v, 1.5 mg/kg) (3 consecutive days doxorubicin (i.v, 1.5 mg/kg) plus cytarabine (50 mg/kg) for 3 days) with or without DARA . 2-3 days after the last treatment, mice were sacrificed and bone marrow, spleen, peripheral blood and plasma were collected for analysis. Flow cytometry was performed to assess percentage of human $CD45^+CD33^+$ cells in the bone marrow **(Figure 5A),** spleen **(Figure 5B)** and peripheral blood **(Figure 5C)** of one AML patient engrafted in NSG mice.

CD38 expression (expressed as mean fluorescence intensity, MFI) was evaluated in the bone marrow **(Figure 6A),** spleen **(Figure 6B)** and peripheral blood **(Figure 6C)** after 5 week treatment with the indicated drugs.

Claims

1. Use of an anti-CD38 antibody in the manufacture of a medicament for the treatment of refractory or relapsed acute myeloid leukemia (AML), wherein 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.

2. The use of claim 1, wherein the anti-CD38 antibody induces killing of AML cells that express CD38 by apoptosis.

3. The use of claim 1 or 2, wherein the anti-CD38 antibody is of IgG1, IgG2, IgG3 or IgG4 isotype.

4. The use of claims 1 or 2, wherein the anti-CD38 antibody comprises the heavy chain variable region (VH) of SEQ ID NO: 4 and the light chain variable region (VL) of SEQ ID NO: 5.

5. The use of claims 1 or 2, wherein the anti-CD38 antibody comprises the heavy chain of SEQ ID NO: 12 and the light chain of SEQ ID NO: 13.

6. The use of any one of claims 2, 4 or 5, wherein AML is AML with at least one genetic abnormality.

7. The use of claim 6, wherein the at least one genetic abnormality is a mutation in a gene selected from the group consisting of fms-related tyrosine kinase 3 (FLT3), nucleophosmin (NPM1), isocitrate dehydrogenase 1(IDH1), isocitrate dehydrogenase 2 (IDH2), DNA (cytosine-5)-methyltransferase 3 (DNMT3A), or CCAAT/enhancer binding protein alpha (CEBPA).

8. The use of claim 7, wherein the mutation in FLT3 is FLT3-ITD, the mutation in IDH2 is R140Q or the mutation in DNMT3A is R882H.

9. The use of any one of claims 1-8, wherein the anti-CD38 antibody is to be administered in combination with at least one second therapeutic agent.

10. The use of claim 9, wherein the at least one second therapeutic agent is cytarabine, daunorubicin, idarubicin, mitoxantrone, hydroxyurea, decitabine, cladribine, fludarabine, topotecan, etoposide[,] 6-thioguanine, corticosteroid, prednisone, dexamethasone, methotrexate, 6-mercaptopurine, azacitidine, arsenic trioxide or all-trans retinoic acid.

11. The use of claim 9, wherein the at least one second therapeutic agent is all-trans retinoic acid, cytarabine, decitabine or doxorubicin.

12. The use of any of the claims 9-11, wherein the anti-CD38 antibody and the at least one second therapeutic agent are to be administered simultaneously, sequentially or separately.

13. The use of any of the claims 9-12, wherein the at least one second therapeutic agent increases surface expression of CD38 on AML cells.

14. The use of any one of claims 1-13, wherein the subject is to be further treated or has been treated with radiotherapy.

15. The use of any one of claims 1-14, wherein the subject is undergoing hematopoietic stem cell transplantation (HSCT).

16. The use of claim 15, wherein the HSCT is allogeneic, autologous or syngeneic.

17. The use of claim 15, wherein the HSCT comprises transplantation of blood stem cells derived from bone marrow, blood or amniotic fluid.

18. The use of any of the claims 1-8, further wherein the subject has been treated with idarubicin, cytrabine or hydroxyurea.