SUBCUTANEOUS FORMULATIONS OF ANTI-CD38 ANTIBODIES AND THEIR USES

SEQUENCE LISTING

This application contains a Sequence Listing submitted via EFS-Web, the entire content incorporated herein by reference in its entirety. The ASCII text file, created on 28 October 2016, is named JBI5070WOPCT_ST25.txt and is 26 kilobytes in size.

FIELD OF THE INVENTION

The present invention relates to subcutaneous formulations of anti-CD38 antibodies and their uses.

BACKGROUND OF THE INVENTION

CD38 is a multifunctional protein having function in receptor-mediated adhesion and signaling as well as mediating calcium mobilization via its ecto-enzymatic activity, catalyzing formation of cyclic ADP-ribose (cADPR) and ADPR. CD38 mediates cytokine secretion and activation and proliferation of lymphocytes (Funaro *et al.,* J Immunol 145:2390-6, 1990; Terhorst *et al.,* Cell 771-80, 1981; Guse *et al.,* Nature 398:70-3, 1999). CD38, via its NAD glycohydrolase activity, also regulates extracellular NAD^+ levels, which have been implicated in modulating the regulatory T-cell compartment (Adriouch *et al.,* 14:1284-92, 2012; Chiarugi *et al.,* Nature Reviews 12:741-52, 2012). In addition to signaling via Ca^{2+}, CD38 signaling occurs via cross-talk with antigen-receptor complexes on T- and B-cells or other types of receptor complexes, e.g., MHC molecules, involving CD38 in several cellular responses, but also in switching and secretion of IgG1. CD38 is expressed on various malignant cells.

Anti-CD38 antibodies are being developed for the treatment of multiple myeloma and other heme malignancies. The antibodies are either injected or infused via the intravenous (IV) route. The amount of antibody that can be administered via the intravenous route is limited by the physico-chemical properties of the antibody, in particularly by its solubility and stability in a suitable liquid formulation and by the volume of the infusion fluid.

Therefore, there is a need for additional anti-CD38 antibody formulations and pharmaceutical compositions.

SUMMARY OF THE INVENTION

The invention provides a pharmaceutical composition comprising an anti-CD38 antibody and a hyaluronidase.

The invention also provides a pharmaceutical composition comprising an anti-CD38 antibody and a hyaluronidase rHuPH20 having the amino acid sequence of SEQ ID NO: 22.

The invention also provides method of treating a cancer in a subject, comprising administering subcutaneously to the subject in need thereof a pharmaceutical composition comprising an anti-CD38 antibody and a hyaluronidase for a time sufficient to treat the cancer.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering subcutaneously to a subject in need thereof the pharmaceutical composition of the invention for a time sufficient to treat the CD38-positive hematological malignancy.

The invention also provides a method of treating a multiple myeloma, comprising administering subcutaneously to a subject in need thereof the pharmaceutical composition of the invention for a time sufficient to treat the multiple myeloma.

The invention also provides a unit dosage form, comprising

an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of from about 1,200 mg to about 5,000 mg;
a hyaluronidase in an amount of from about 30,000 U to about 45,000 U;
histidine at a concentration of from about 5 mM to about 15 mM;
sorbitol at a concentration of from about 100 mM to about 300 mM;
PS-20 at a concentration of from about 0.01% w/v to about 0.04 % w/v; and
methionine at a concentration of from about 1 mg/mL to about 2 mg/mL, at a pH of about 5.5.

The invention also provides a unit dosage form of claim 74, comprising

the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of about 1,800 mg;
a hyaluronidase in an amount of about 30,000 U;
histidine at a concentration of about 10 mM;
sorbitol at a concentration of about 300 mM;

PS-20 at a concentration of about 0.04 % w/v; and

methionine at a concentration of about 1 mg/mL, at a pH of about 5.5.

The invention also provides a container comprising the unit dosage form of the invention.

The invention also provides a container comprising the pharmaceutical composition of the invention.

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 GenBank accession number NP_001766 and in SEQ ID NO: 1. It is well known that CD38 is a single pass type II membrane protein with amino acid residues 1-21 representing the cytosolic domain, amino acid residues 22-42 representing the transmembrane domain, and residues 43-300 representing the extracellular domain of CD38.

SEQ ID NO: 1

MANCEFSPVSGDKPCCRLSRRAQLCLGVSILVLILVVVLAVVVPRWRQQWSGPGT
TKRFPETVLARCVKYTEIHPEMRHVDCQSVWDAFKGAFISKHPCNITEEDYQPLM
KLGTQTVPCNKILLWSRIKDLAHQFTQVQRDMFTLEDTLLGYLADDLTWCGEFN
TSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVVHVMLNGSRSKIFDK
NSTFGSVEVHNLQPEKVQTLEAWVIHGGREDSRDLCQDPTIKELESIISKRNIQFSC
KNIYRPDKFLQCVKNPEDSSCTSEI

"Antibodies" is meant in a broad sense and includes immunoglobulin molecules including monoclonal antibodies including murine, human, humanized and chimeric monoclonal antibodies, antigen-binding fragments, bispecific or multispecific antibodies, dimeric, tetrameric or multimeric antibodies, single chain antibodies, domain antibodies and any other modified configuration of the immunoglobulin molecule that comprises an antigen binding site of the required specificity. "Full length antibodies" are comprised of two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds as well as multimers thereof (for example IgM). Each heavy chain is comprised of a heavy chain variable region (VH) and a heavy chain constant region (comprised of domains CH1,

hinge CH2 and CH3). Each light chain is comprised of a light chain variable region (VL) and a light chain constant region (CL). The VH and the VL regions may be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with framework regions (FR). Each VH and VL is composed of three CDRs and four FR segments, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, and FR4.

"Complementarity determining regions (CDR)" are "antigen binding sites" in an antibody. CDRs may be defined using various terms: (i) Complementarity Determining Regions (CDRs), three in the VH (HCDR1, HCDR2, HCDR3) and three in the VL (LCDR1, LCDR2, LCDR3) are based on sequence variability (Wu and Kabat, J Exp Med 132:211-50, 1970; Kabat *et al.,* Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, Md., 1991). (ii) "Hypervariable regions", "HVR", or "HV", three in the VH (H1, H2, H3) and three in the VL (L1, L2, L3) refer to the regions of an antibody variable domains which are hypervariable in structure as defined by Chothia and Lesk (Chothia and Lesk, Mol Biol 196:901-17, 1987). 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. The term "CDR", "HCDR1", "HCDR2", "HCDR3", "LCDR1", "LCDR2" and "LCDR3" as used herein includes CDRs defined by any of the methods described *supra,* Kabat, Chothia or IMGT, unless otherwise explicitly stated in the specification.

Immunoglobulins may be assigned to five major classes, 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 can be assigned to one of two clearly distinct types, namely kappa (κ) and lambda (λ), based on the amino acid sequences of their constant domains.

"Antigen-binding fragment" refers to a portion of an immunoglobulin molecule that retains the antigen binding properties of the parental full length antibody. Exemplary antigen-binding fragments are as heavy chain complementarity determining regions (HCDR) 1, 2 and/or 3, light chain complementarity determining regions (LCDR) 1, 2 and/or 3, a heavy chain variable region (VH), or a light chain variable region (VL), Fab,

F(ab')2, Fd and Fv fragments as well as domain antibodies (dAb) consisting of either one VH domain or one VL domain. VH and VL domains may be linked together via a synthetic linker to form various types of single chain antibody designs in which the VH/VL domains pair intramolecularly, or intermolecularly in those cases when the VH and VL domains are expressed by separate chains, to form a monovalent antigen binding site, such as single chain Fv (scFv) or diabody; described for example in Int. Pat. Publ. No. WO1998/44001, Int. Pat. Publ. No. WO1988/01649; Int. Pat. Publ. No. WO1994/13804; Int. Pat. Publ. No. WO1992/01047.

"Monoclonal antibody" refers to an antibody population with single amino acid composition in each heavy and each light chain, except for possible well known alterations such as removal of C-terminal lysine from the antibody heavy chain. Monoclonal antibodies typically bind one antigenic epitope, except that multispecific monoclonal antibodies bind two or more distinct antigens or epitopes. Bispecific monoclonal antibodies bind two distinct antigenic epitopes. Monoclonal antibodies may have heterogeneous glycosylation within the antibody population. Monoclonal antibody may be monospecific or multispecific, or monovalent, bivalent or multivalent. A multispecific antibody, such as a bispecific antibody or a trispecific antibody is included in the term monoclonal antibody.

"Isolated antibody" refers to an antibody or an antigen-binding fragment thereof that is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody specifically binding human CD38 is substantially free of antibodies that specifically bind antigens other than human CD38). In case of a bispecific antibody, the bispecific antibody specifically binds two antigens of interest, and is substantially free of antibodies that specifically bind antigens other that the two antigens of interest. "Isolated antibody" encompasses antibodies that are isolated to a higher purity, such as antibodies that are 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% pure.

"Humanized antibodies" refers to antibodies 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 intentionally introduced mutations in the framework regions so that the framework may not be an exact copy of expressed human immunoglobulin or germline gene sequences.

"Human antibodies" refers to antibodies having heavy and light chain variable regions in which both the framework and the antigen binding site are derived from sequences of human origin. If the antibody contains a constant region or a portion of the constant region, the constant region also is derived from sequences of human origin.

A human antibody comprises heavy or light chain variable regions that are derived from sequences of human origin if the variable regions of the antibody are obtained from a system that uses human germline immunoglobulin or rearranged immunoglobulin genes. Such exemplary systems are human immunoglobulin gene libraries displayed on phage, and transgenic non-human animals such as mice or rats carrying human immunoglobulin loci as described herein. A human antibody typically contains amino acid differences when compared to the human germline or rearranged immunoglobulin sequences due to, for example naturally occurring somatic mutations, intentional introduction of substitutions into the framework or antigen binding site and amino acid changes introduced during cloning and VDJ recombination in non-human animals. Typically, a human antibody is at least about 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% identical in amino acid sequence to an amino acid sequence encoded by a human germline or rearranged immunoglobulin gene. In some cases, a human antibody may contain consensus framework sequences derived from human framework sequence analyses, for example as described in Knappik *et al.*, J Mol Biol 296:57-86, 2000, or synthetic HCDR3 incorporated into human immunoglobulin gene libraries displayed on phage, for example as described in Shi *et al.,* J Mol Biol 397:385-96, 2010 and Int. 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.

"Recombinant" includes antibodies and other proteins that are prepared, expressed, created or isolated by recombinant means.

"Epitope" refers to a portion of an antigen to which an antibody specifically binds. Epitopes typically consist of chemically active (such as polar, non-polar or hydrophobic) surface groupings of moieties such as amino acids or polysaccharide side chains and may have specific three-dimensional structural characteristics, as well as specific charge characteristics. An 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.

"Multispecific" refers to an antibody that specifically binds at least two distinct antigens or two distinct epitopes within the antigens, for example three, four or five distinct antigens or epitopes.

"Bispecific" refers to an antibody that specifically binds two distinct antigens or two distinct epitopes within the same antigen. The bispecific antibody may have cross-reactivity to other related antigens or can bind an epitope that is shared between two or more distinct antigens.

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

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

"Pharmaceutical composition" refers to a product that results from combining an anti-CD38 antibody and a hyaluronidase and includes both fixed and non-fixed combinations. Pharmaceutical composition typically includes a pharmaceutically acceptable carrier. "Fixed combinations" refers to a single pharmaceutical composition comprising the anti-CD38 antibody and the hyaluronidase administered simultaneously in the form of a single entity or dosage. "Non-fixed combination" refers to separate pharmaceutical compositions of the anti-CD38 antibody and the hyaluronidase or unit dosage forms administered as separate entities either simultaneously, concurrently or sequentially with no specific intervening time limits, wherein such administration provides effective levels of the two compounds in the body of the subject.

"Pharmaceutically acceptable carrier" refers to an ingredient in a pharmaceutical composition, other than an active ingredient, which is nontoxic to a subject. A pharmaceutically acceptable carrier includes, but is not limited to, a buffer, excipient, stabilizer, or preservative.

"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 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, lack of metastasis, 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 well as those subjects prone to have the physiological change or disease.

"Therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve the 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 in a tumor burden, arrested or slowed growth of a tumor, and/or absence of metastasis of cancer cells to other locations in the body.

"Inhibits growth" (e.g. referring to tumor cells) refers to a measurable decrease in the tumor cell growth or tumor tissue *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 tumor cells or tumor tissue in the absence of the therapeutic or the combination of therapeutic drugs. Inhibition of growth of a tumor cell or tumor tissue *in vitro* or *in vivo* may be at least about 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 99%, or 100%.

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

lymphoproliferative disorder, light chain amyloidosis, Waldenstrom's macroglobulinemia, plasma cell leukemias and anaplastic large-cell lymphoma (ALCL).

"About" means within an acceptable error range for the particular value as determined by one of ordinary skill in the art, which will depend in part on how the value is measured or determined, i.e., the limitations of the measurement system. Unless explicitly stated otherwise within the Examples or elsewhere in the Specification in the context of a particular assay, result or embodiment, "about" means within one standard deviation per the practice in the art, or a range of up to 5%, whichever is larger.

Pharmaceutical compositions

The invention provides a pharmaceutical composition comprising an anti-CD38 antibody and a hyaluronidase.

Hyaluronidase is an enzyme that degrades hyaluronic acid (EC 3.2.1.35) and lowers the viscosity of hyaluronan in the extracellular matrix, thereby increasing tissue permeability. Enzymatic activity of hyaluronidase, including rHuPH20, can be defined by units per mL (U/mL) or by total enzyme activity in a particular formulation (U) as further explained below.

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

The standard definition for one unit (U) of enzyme activity is the amount of enzyme that catalyzes the reaction of a defined amount of substrate per unit time, such as one μmole or one nmol substrate per minute. Techniques for determing activity of hyaluronidase preparations are known in the art and activity of hyaluronidase preparations are typically expressed as either USP units or Units (hereinafter "Units"). An exemplary method for determing activity can be found in United States Pat. No. 7,767,429.

Hyaluronidase activity refers to the ability to enzymatically catalyze the cleavage of hyaluronic acid. The United States Pharmacopeia (USP) XXII provides an assay for hyaluronidase where hyaluronidase activity is indirectly determined by measuring the amount of higher molecular weight hyaluronic acid, or hyaluronan, (HA) substrate remaining after the enzyme is allowed to react with the HA for 30 min at 37° C. (USP XXII-NF XVII (1990) 644-645 United States Pharmacopeia Convention, Inc, Rockville, Md.). A Reference Standard solution can be used in an assay to ascertain the relative activity, in Units, of any hyaluronidase. In vitro assays to determine the hyaluronidase

activity of hyaluronidases, such as soluble rHuPH20, are known in the art and described herein. Exemplary assays include the microturbidity assay described below (see e.g. Example 3) that measures cleavage of hyaluronic acid by hyaluronidase indirectly by detecting the insoluble precipitate formed when the uncleaved hyaluronic acid binds with serum albumin. Reference Standards can be used, for example, to generate a standard curve to determine the activity in Units of the hyaluronidase being tested.

The pharmaceutical composition is useful for subcutaneous administration of the anti-CD38 antibody to a subject in need of anti-CD38 antibody therapy, such as a subject having a cancer, for example a CD38-positive hematological malignancy. Without wishing to be bound by any particular theory, subcutaneous administration of the anti-CD38 antibody may have reduced infusion related reaction and achieve improved response rates when compared to the intravenous administration of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition is a fixed combination.

In some embodiments, the pharmaceutical composition is a non-fixed combination.

In some embodiments, the pharmaceutical composition comprises from about 1 mg/mL to about 180 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 10 mg/mL to about 180 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 20 mg/mL to about 160 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 20 mg/mL to about 140 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 20 mg/mL to about 120 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 40 mg/mL to about 120 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 60 mg/mL to about 120 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 80 mg/mL to about 120 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 100 mg/mL to about 120 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 1 mg/mL, about 5 mg/mL, about 10 mg/mL, about 15 mg/mL, about 20 mg/mL, about 30 mg/mL, about 40 mg/mL, about 50 mg/mL, about 60 mg/mL, about 70 mg/mL, about 80 mg/mL, about 90 mg/mL, about 100 mg/mL, about 110 mg/mL, about 120 mg/mL, about 130 mg/mL, about 140 mg/mL, about 150 mg/mL, about 160 mg/mL, about 170 mg/mL or about 180 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 20 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 100 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 120 mg/mL of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 50 U/mL to about 5,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 500 U/mL to about 5,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 1,000 U/mL to about 5,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 2,000 U/mL to about 5,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 50 U/mL to about 2,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 500 U/mL to about 2,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 1,000 U/mL to about 2,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 500 U/mL, about 600 U/mL, about 700 U/mL, about 800 U/mL, about 900 U/mL, about 1,000 U/mL, about 1,100 U/mL, about 1,200 U/mL, about 1,300 U/mL, about 1,400 U/mL, about 1,500 U/mL, about 1,600 U/mL, about 1,700 U/mL, about 1,800 U/mL, about 1,900 U/mL, about 2,000 U/mL, about 2,100 U/mL, about 2,200 U/mL, about 2,300 U/mL,

about 2,400 U/mL, about 2,500 U/mL, about 2,600 U/mL, about 2,700 U/mL, about 2,800 U/mL, about 2,900 U/mL, about 3,000 U/mL, about 3,100 U/mL, about 3,200 U/mL, about 3,300 U/mL, about 3,400 U/mL, about 3,500 U/mL, about 3,600 U/mL, about 3,700 U/mL, about 3,800 U/mL, about 3,900 U/mL, about 4,000 U/mL, about 4,100 U/mL, about 4,200 U/mL, about 4,300 U/mL, about 4,400 U/mL, about 4,500 U/mL, about 4,600 U/mL, about 4,700 U/mL, about 4,800 U/mL, about 4,900 U/mL or about 5,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 500 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 5,000 U/mL of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 1,200 mg to about 5,000 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 1,200 mg to about 2,400 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 1,200 mg to about 1,800 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 1,200 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 1,400 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 1,600 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 1,800 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 2,000 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 2,200 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 2,400 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 2,600 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 2,800 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 3,000 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 3,500 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 4,000 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 4,500 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises about 5,000 mg of the anti-CD38 antibody.

In some embodiments, the pharmaceutical composition comprises from about 750 U to about 75,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 7,500 U to about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises from about 30,000 U to about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 7,500 U, about 8,000 U, about 8,500 U, about 9,000 U, about 10,000 U, about 15,000 U, about 20,000 U, about 21,000 U, about 22,000 U, about 23,000 U, about 24,000 U, about 25,000 U, about 26,000 U, about 27,000 U, about 28,000 U, about 29,000 U, about 30,000 U, about 31,000 U, about 32,000 U, about 33,000 U, about 34,000 U, about 35,000 U, about 36,000 U, about 37,000 U, about 38,000 U, about 39,000 U, about 40,000 U, about 41,000 U, about 42,000 U, about 43,000 U, about 44,000 U, about 45,000 U, about 46,000 U, about 47,000 U, about 48,000 U, about 49,000 U, about 50,000 U, about 55,000 U, about 60,000 U, about 65,000 U, about 70,000 U or about 75,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 5,000 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 5,000 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 3,000 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 3,000 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,800 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,800 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,600 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,600 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,400 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,400 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,200 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,200 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,000 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 2,000 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 1,800 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 1,800 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 1,600 mg of the anti-CD38 antibody and about 30,000 U of the hyaluronidase.

In some embodiments, the pharmaceutical composition comprises about 1,600 mg of the anti-CD38 antibody and about 45,000 U of the hyaluronidase.

In some embodiments, the hyaluronidase is rHuPH20 having the amino acid sequence of SEQ ID NO: 22.

In some embodiments, the anti-CD38 antibody in the pharmaceutical composition 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.

In some embodiments, the anti-CD38 antibody in the pharmaceutical composition binds at least to the region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1).

In some embodiments, the anti-CD38 antibody in the pharmaceutical composition comprises a heavy chain complementarity determining region 1 (HCDR1), a HCDR2, a HCDR3, a light chain complementarity determining region 1 (LCDR1), a LCDR2 and a LCDR3 of SEQ ID NOs: 6, 7 and 8, 9, 10 and 11, respectively.

In some embodiments, the anti-CD38 antibody in the pharmaceutical composition comprises a heavy chain variable region (VH) that is 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 4 and a light chain variable region (VL) that is 95%, 96%, 97%, 98%, 99% or 100% identical to the amino acid sequence of SEQ ID NO: 5.

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

In some embodiments, the anti-CD38 antibody in the pharmaceutical composition comprises a heavy chain of SEQ ID NO: 12 and a light chain of SEQ ID NO: 13.

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

Other exemplary anti-CD38 antibodies that may be used in the pharmaceutical compositions and the methods of the invention are:

mAb003 comprising the VH and the VL sequences of SEQ ID NOs: 14 and 15, respectively and described in U.S. Pat. No. 7,829,693. The VH and the VL of mAb003 may be expressed as IgG1/κ;

mAb024 comprising the VH and the VL sequences of SEQ ID NOs: 16 and 17, respectively, described in U.S. Pat. No. 7,829,693. The VH and the VL of mAb024 may be expressed as IgG1/κ;

MOR-202 (MOR-03087) comprising the VH and the VL sequences of SEQ ID NOs: 18 and 19, respectively, described in US. Pat. No. 8,088,896. The VH and the VL of MOR-202 may be expressed as IgG1/κ; or

Isatuximab; comprising the VH and the VL sequences of SEQ ID NOs: 20 and 21, respectively, described in U.S. Pat. No. 8,153,765. The VH and the VL of Isatuximab may be expressed as IgG1/κ.

SEQ ID NO: 14

QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPGQGLEWMGRVIPF
LGIANSAQKFQGRVTITADKSTSTAYMDLSSLRSEDTAVYYCARDDIAALGPFDY
WGQGTLVTVSSAS

SEQ ID NO: 15

DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQS
GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQYNSYPRTFGQGTKVEIK

SEQ ID NO: 16

EVQLVQSGAEVKKPGESLKISCKGSGYSFSNYWIGWVRQMPGKGLEWMGIIYPH
DSDARYSPSFQGQVTFSADKSISTAYLQWSSLKASDTAMYYCARHVGWGSRYW
YFDLWGRGTLVTVSS

SEQ ID NO: 17
EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAPGLLIYDASNRAS
GIPARFSGSGSGTDFTLTISSLEPEDFAVYYCQQRSNWPLTFGGGTKVEIK

SEQ ID NO: 18
QVQLVESGGGLVQPGGSLRLSCAASGFTFSSYYMNWVRQAPGKGLEWVSGISGD
PSNTYYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDLPLVYTGFA
YWGQGTLVTVSS

SEQ ID NO: 19
DIELTQPPSVSVAPGQTARISCSGDNLRHYYVYWYQQKPGQAPVLVIYGDSKRPS
GIPERFSGSNSGNTATLTISGTQAEDEADYYCQTYTGGASLVFGGGTKLTVLGQ

SEQ ID NO 20:
QVQLVQSGAEVAKPGTSVKLSCKASGYTFTDYWMQWVKQRPGQGLEWIGT
IYPGDGDTGYAQKFQGKATLTADKSSKTVYMHLSSLASEDSAVYYCARGD
YYGSNSLDYWGQGTSVTVSS

SEQ ID NO: 21:
DIVMTQSHLSMSTSLGDPVSITCKASQDVSTVVAWYQQKPGQSPRRLIYS
ASYRYIGVPDRFTGSGAGTDFTFTISSVQAEDLAVYYCQQHYSPPYTFGG
GTKLEIK

Other exemplary anti-CD38 antibodies that may be used in the pharmaceutical compositions of the invention are 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.

An exemplary anti-CD38 antibody that may be used in the pharmaceutical compositions of the invention is daratumumab. Daratumumab comprises the heavy chain

variable region (VH) and the light chain variable region (VL) amino acid sequences shown in SEQ ID NOs: 4 and 5, respectively, the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 6, 7 and 8, respectively, the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 9, 10 and 11, respectively, and is of IgG1/κ subtype and described in U.S. Pat. No. 7,829,693. Daratumumab heavy chain amino acid sequence is shown in SEQ ID NO: 12 and light chain amino acid sequence shown in SEQ ID NO: 13.

The invention also provides a pharmaceutical composition comprising an anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 and a hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a pharmaceutical composition comprising an anti-CD38 antibody comprising the HCDR1, the HCDR2 and the HCDR3 of SEQ ID NOs: 6, 7 and 8, respectively, and the LCDR1, the LCDR2 and the LCDR3 of SEQ ID NOs: 9, 10 and 11, respectively and the hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a pharmaceutical composition comprising between about 1,200 mg – 1,800 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and between about 30,000 U – 45,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a pharmaceutical composition comprising about 1,800 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 30,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a pharmaceutical composition comprising about 1,800 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 45,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a pharmaceutical composition comprising about 1,600 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 30,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein

the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a pharmaceutical composition comprising about 1,600 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 45,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a pharmaceutical composition comprising about 1,200 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 30,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a pharmaceutical composition comprising about 1,200 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 45,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

SEQ ID NO: 22

MGVLKFKHIFFRSFVKSSGVSQIVFTFLLIPCCLTLNFRAPPVIPNVPFLWAWNAPS
EFCLGKFDEPLDMSLFSFIGSPRINATGQGVTIFYVDRLGYYPYIDSITGVTVNGGIP
QKISLQDHLDKAKKDITFYMPVDNLGMAVIDWEEWRPTWARNWKPKDVYKNRS
IELVQQQNVQLSLTEATEKAKQEFEKAGKDFLVETIKLGKLLRPNHLWGYYLFPD
CYNHHYKKPGYNGSCFNVEIKRNDDLSWLWNESTALYPSIYLNTQQSPVAATLY
VRNRVREAIRVSKIPDAKSPLPVFAYTRIVFTDQVLKFLSQDELVYTFGETVALGA
SGIVIWGTLSIMRSMKSCLLLDNYMETILNPYIINVTLAAKMCSQVLCQEQGVCIR
KNWNSSDYLHLNPDNFAIQLEKGGKFTVRGKPTLEDLEQFSEKFYCSCYSTLSCK
EKADVKDTDAVDVCIADGVCIDAFLKPPMETEEPQIFYNASPSTLSATMFIVSILFLI
ISSVASL

Anti-CD38 antibodies used in the pharmaceutical compositions of the invention, may also be selected *de novo* from, e.g., a phage display library, where the phage is engineered to express human immunoglobulins or portions thereof such as Fabs, single

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

Antibodies may be evaluated for their competition with a reference antibody such as the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 for binding to CD38 using known *in vitro* methods. In an exemplary method, CHO cells recombinantly expressing CD38 may be incubated with unlabeled reference antibody for 15 min at 4°C, followed by incubation with an excess of fluorescently labeled test antibody for 45 min at 4°C. After washing in PBS/BSA, fluorescence may be measured by flow cytometry using standard methods. In another exemplary method, extracellular portion of human CD38 may be coated on the surface of an ELISA plate. Excess of unlabeled reference antibody may be added for about 15 minutes and subsequently biotinylated test antibodies may be added. After washes in PBS/Tween, binding of the test biotinylated antibody may be detected using horseradish peroxidase (HRP)-conjugated streptavidine and the signal detected using standard methods. It is readily apparent that in the competition assays, the reference antibody may be labelled and the test antibody unlabeled. The test antibody competes with the reference antibody when the reference antibody inhibits binding of the test antibody, or the test antibody inhibits binding of the reference antibody by at least 80%, for example 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% 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 region SKRNIQFSCKNIYR (SEQ ID NO: 2) and the region EKVQTLEAWVIHGG (SEQ ID NO: 3) of human CD38 (SEQ ID NO: 1) may be generated for example by immunizing mice with peptides having the amino acid sequences shown in SEQ ID NOs: 2 and 3 using standard methods and those described herein, and characterizing the obtained antibodies for binding to the peptides using for example ELISA or mutagenesis studies.

The invention also provides a pharmaceutical composition comprising an anti-CD38 antibody comprising the HCDR1, the HCDR2, the HCDR3, the LCDR1, the LCDR2, and the LCDR3 sequences of:

a. the VH of SEQ ID NO: 14 and the VL of SEQ ID NO: 15;
b. the VH of SEQ ID NO: 16 and the VL of SEQ ID NO: 17;
c. the VH of SEQ ID NO: 18 and the VL of SEQ ID NO: 19; or
d. the VH of SEQ ID NO: 20 and the VL of SEQ ID NO: 21, and the hyaluronidase rHuPH20 of SEQ ID NO: 22.

The invention also provides a pharmaceutical composition comprising an anti-CD38 antibody comprising

a. the VH of SEQ ID NO: 14 and the VL of SEQ ID NO: 15;
b. the VH of SEQ ID NO: 16 and the VL of SEQ ID NO: 17;
c. the VH of SEQ ID NO: 18 and the VL of SEQ ID NO: 19; or
d. the VH of SEQ ID NO: 20 and the VL of SEQ ID NO: 21, and the hyaluronidase rHuPH20 of SEQ ID NO: 22.

The pharmaceutical compositions of the invention further comprise a pharmaceutically acceptable carrier. Exemplary pharmaceutically acceptable carriers are solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like that are physiologically compatible, such as salts, buffers, antioxidants, saccharides, aqueous or non-aqueous carriers, preservatives, wetting agents, surfactants or emulsifying agents, or combinations thereof.

Exemplary buffers that may be used are acetic acid, citric acid, formic acid, succinic acid, phosphoric acid, carbonic acid, malic acid, aspartic acid, histidine, boric acid, Tris buffers, HEPPSO and HEPES.

Exemplary antioxidants that may be used are ascorbic acid, methionine, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite, lecithin, citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol and tartaric acid.

Exemplary amino acids that may be used are histidine, isoleucine, methionine, glycine, arginine, lysine, L-leucine, tri-leucine, alanine, glutamic acid, L-threonine, and 2-phenylamine.

Exemplary surfactants that may be used are polysorbates (e.g., polysorbate-20 or polysorbate-80); polyoxamers (e.g., poloxamer 188); Triton; sodium octyl glycoside; lauryl-, myristyl-, linoleyl-, or stearyl-sulfobetaine; lauryl-, myristyl-, linoleyl- or stearyl-sarcosine; linoleyl-, myristyl-, or cetyl-betaine; lauroamidopropyl-, cocamidopropyl-, linoleamidopropyl-, myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-betaine (e.g., lauroamidopropyl); myristamidopropyl-, palmidopropyl-, or isostearamidopropyl-dimethylamine; sodium methyl cocoyl-, or disodium methyl oleyl-taurate; and the MONAQUA™ series (Mona Industries, Inc., Paterson, N.J.), polyethyl glycol, polypropyl glycol, and copolymers of ethylene and propylene glycol (e.g., PLURONICS™, PF68, etc).

Exemplary preservatives that may be used are phenol, m-cresol, p-cresol, o-cresol, chlorocresol, benzyl alcohol, phenylmercuric nitrite, phenoxyethanol, formaldehyde, chlorobutanol, magnesium chloride, alkylparaben (methyl, ethyl, propyl, butyl and the like), benzalkonium chloride, benzethonium chloride, sodium dehydroacetate and thimerosal, or mixtures thereof.

Exemplary saccharides that may be used are monosaccharides, disaccharides, trisaccharides, polysaccharides, sugar alcohols, reducing sugars, nonreducing sugars such as glucose, sucrose, trehalose, lactose, fructose, maltose, dextran, glycerin, dextran, erythritol, glycerol, arabitol, sylitol, sorbitol, mannitol, mellibiose, melezitose, raffinose, mannotriose, stachyose, maltose, lactulose, maltulose, glucitol, maltitol, lactitol or iso-maltulose.

Exemplary salts that may be used are acid addition salts and base addition salts. Acid addition salts include those derived from nontoxic inorganic acids, such as hydrochloric, nitric, phosphoric, sulfuric, hydrobromic, hydroiodic, phosphorous and the like, as well as from nontoxic organic acids such as aliphatic mono- and dicarboxylic acids, phenyl-substituted alkanoic acids, hydroxy alkanoic acids, aromatic acids, aliphatic and aromatic sulfonic acids and the like. Base addition salts include those derived from

alkaline earth metals, such as sodium, potassium, magnesium, calcium and the like, as well as from nontoxic organic amines, such as N,N′-dibenzylethylenediamine, N-methylglucamine, chloroprocaine, choline, diethanolamine, ethylenediamine, procaine and the like. An exemplary salt is sodium chloride.

The amounts of pharmaceutically acceptable carrier(s) in the pharmaceutical compositions may be determined experimentally based on the activities of the carrier(s) and the desired characteristics of the formulation, such as stability and/or minimal oxidation.

In some embodiments, the pharmaceutical composition comprises acetic acid.

In some embodiments, the pharmaceutical composition comprises acetic acid at a concentration of from about 1 mM to about 50 mM.

In some embodiments, the pharmaceutical composition comprises acetic acid at a concentration of from about 10 mM to about 40 mM.

In some embodiments, the pharmaceutical composition comprises acetic acid at a concentration of about 10 mM, about 15 mM, about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM or about 50 mM.

In some embodiments, the pharmaceutical composition comprises acetic acid at a concentration of about 25 mM.

In some embodiments, the pharmaceutical composition comprises sodium chloride (NaCl).

In some embodiments, the pharmaceutical composition comprises NaCl at a concentration of from about 20 mM to about 100 mM.

In some embodiments, the pharmaceutical composition comprises NaCl at a concentration of from about 40 mM to about 80 mM.

In some embodiments, the pharmaceutical composition comprises NaCl at a concentration of about 20 mM, about 25 mM, about 30 mM, about 35 mM, about 40 mM, about 45 mM, about 50 mM, about 55 mM, about 60 mM, about 65 mM, about 70 mM, about 75 mM, about 80 mM, about 85 mM, about 90 mM, about 95 mM or about 100 mM.

In some embodiments, the pharmaceutical composition comprises NaCl at a concentration of about 60 mM.

In some embodiments, the pharmaceutical composition comprises saccharide.

In some embodiments, saccharide is sucrose.

In some embodiments, saccharide is sorbitol.

In some embodiments, saccharide is mannitol.

In some embodiments, the pharmaceutical composition comprises saccharide at a concentration of from about 50 mM to about 500 mM.

In some embodiments, the pharmaceutical composition comprises saccharide at a concentration of from about 50 mM to about 450 mM.

In some embodiments, the pharmaceutical composition comprises saccharide at a concentration of from about 50 mM to about 400 mM.

In some embodiments, the pharmaceutical composition comprises saccharide at a concentration of from about 50 mM to about 350 mM.

In some embodiments, the pharmaceutical composition comprises saccharide at a concentration of from about 100 mM to about 350 mM.

In some embodiments, the pharmaceutical composition comprises saccharide at a concentration of from about 100 mM to about 300 mM.

In some embodiments, the pharmaceutical composition comprises saccharide at a concentration of about 100 mM , about 110 mM, about 120 mM, about 130 mM, about 140 mM, about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, about 200 mM, about 210 mM, about 220 mM, about 230 mM, about 240 mM, about 250 mM, about 260 mM, about 270 mM, about 280 mM, about 290 mM, about 300 mM, about 310 mM, about 320 mM, about 330 mM, about 340 mM, about 350 mM, about 360 mM, about 370 mM, about 380 mM, about 390 mM, about 400 mM, about 410 mM, about 420 mM, about 430 mM, about 440 mM, about 450 mM, about 460 mM, about 470 mM, about 480 mM, about 490 mM or about 500 mM.

In some embodiments, the pharmaceutical composition comprises mannitol.

In some embodiments, the pharmaceutical composition comprises mannitol at a concentration of from about 100 mM to about 180 mM.

In some embodiments, the pharmaceutical composition comprises mannitol at a concentration of from about 120 mM to about 160 mM.

In some embodiments, the pharmaceutical composition comprises mannitol at a concentration of about 100 mM, about 105 mM, about 110 mM, about 115 mM, about 120 mM, about 125 mM, about 130 mM, about 135 mM, about 140 mM, about 145 mM, about 150 mM, about 155 mM, about 160 mM, about 165 mM, about 170 mM, about 175 mM or about 180 mM.

In some embodiments, the pharmaceutical composition comprises mannitol at a concentration of about 140 mM.

In some embodiments, the pharmaceutical composition comprises polysorbate.

In some embodiments, the pharmaceutical composition comprises polysorbate-20 (PS-20).

In some embodiments, the pharmaceutical composition comprises polysorbate-20 (PS-20) at a concentration of from about 0.01% w/v to about 0.1% w/v.

In some embodiments, the pharmaceutical composition comprises polysorbate-20 (PS-20) at a concentration of from about 0.01% w/v to about 0.08% w/v.

In some embodiments, the pharmaceutical composition comprises polysorbate-20 (PS-20) at a concentration of from about 0.01% w/v to about 0.04% w/v.

In some embodiments, the pharmaceutical composition comprises polysorbate-20 (PS-20) at a concentration of about 0.01% w/v, 0.02% w/v, 0.03% w/v, 0.04% w/v, 0.05% w/v, 0.06% w/v, 0.07% w/v, 0.08% w/v, 0.09% w/v or 0.1% w/v.

The invention also provides a pharmaceutical composition comprising

from about 20 mg/mL to about 120 mg/mL of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in about 25 mM acetic acid, about 60 mM sodium chloride, about 140 mM mannitol and about 0.04% w/v polysorbate-20 (PS-20); at pH about 5.5; and

about from 30,000 U to about 45,000 U of the hyaluronidase in 10 mM L-Histidine, 130 mM NaCl, 10 mM L-Methionine, 0.02% Polysorbate 80, pH 6.5.

In some embodiments, the hyaluronidase is rHuPH20 (SEQ ID NO: 22).

In some embodiments, the pharmaceutical composition is a non-fixed combination.

The invention also provides a pharmaceutical composition comprising

about 20 mg/mL of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in about 25 mM acetic acid, about 60 mM sodium chloride, about 140 mM mannitol and about 0.04% w/v polysorbate-20 (PS-20); at pH about 5.5; and

about 30,000 U of the hyaluronidase in 10 mM L-Histidine, 130 mM NaCl, 10 mM L-Methionine, 0.02% Polysorbate 80, pH 6.5.

In some embodiments, the hyaluronidase is rHuPH20 (SEQ ID NO: 22).

In some embodiments, the pharmaceutical composition is a non-fixed combination.

The invention also provides a pharmaceutical composition comprising

about 20 mg/mL of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in about 25 mM acetic acid, about 60 mM sodium chloride, about 140 mM mannitol and about 0.04% w/v polysorbate-20 (PS-20); at pH about 5.5; and

about 45,000 U of the hyaluronidase in 10 mM L-Histidine, 130 mM NaCl, 10 mM L-Methionine, 0.02% Polysorbate 80, pH 6.5.

In some embodiments, the hyaluronidase is rHuPH20 (SEQ ID NO: 22).

In some embodiments, the pharmaceutical composition is a non-fixed combination.

In some embodiments, the pharmaceutical composition comprises histidine.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of from about 1 mM to about 50 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of from about 5 mM to about 50 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of from about 5 mM to about 30 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of from about 5 mM to about 20 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of from about 5 mM to about 15 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of from about 5 mM to about 10 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of about 1 mM, about 2 mM, about 3 mM, about 4 mM, about 5 mM, about 6 mM, about 7 mM, about 8 mM, about 9 mM, about 10 mM, about 11 mM, about 12 mM, about 13 mM, about 14 mM, about 15 mM, about 16 mM, about 17 mM, about 18 mM, about 19 mM, about 20 mM, about 21 mM, about 22 mM, about 23 mM, about 24 mM, about 25 mM, about 26 mM, about 27 mM, about 28 mM, about 29 mM, about 30 mM, about 31 mM, about 32 mM, about 33 mM, about 34 mM, about 35 mM, about 36

mM, about 37 mM, about 38 mM, about 39 mM, about 40 mM, about 41 mM, about 42 mM, about 43 mM, about 44 mM, about 45 mM, about 46 mM, about 47 mM, about 48 mM, about 49 mM or about 50 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of about 5 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of about 10 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of about 15 mM.

In some embodiments, the pharmaceutical composition comprises histidine at a concentration of about 20 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of from about 50 mM to about 500 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of from about 50 mM to about 450 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of from about 50 mM to about 400 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of from about 50 mM to about 350 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of from about 100 mM to about 350 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of from about 100 mM to about 300 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 100 mM , about 110 mM, about 120 mM, about 130 mM, about 140 mM, about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, about 200 mM, about 210 mM, about 220 mM, about 230 mM, about 240 mM, about 250 mM, about 260 mM, about 270 mM, about 280 mM, about 290 mM, about 300 mM, about 310 mM, about 320 mM, about 330 mM, about 340 mM, about 350 mM, about 360 mM, about 370 mM, about 380 mM, about 390 mM, about 400 mM, about 410 mM, about 420 mM, about 430 mM, about 440 mM, about 450 mM, about 460 mM, about 470 mM, about 480 mM, about 490 mM or about 500 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 50 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 100 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 150 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 200 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 250 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 300 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 350 mM.

In some embodiments, the pharmaceutical composition comprises sorbitol at a concentration of about 400 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of from about 50 mM to about 500 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of from about 50 mM to about 450 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of from about 50 mM to about 400 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of from about 50 mM to about 350 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of from about 100 mM to about 350 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of from about 100 mM to about 200 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 100 mM , about 110 mM, about 120 mM, about 130 mM, about 140 mM, about 150 mM, about 160 mM, about 170 mM, about 180 mM, about 190 mM, about 200 mM, about 210 mM, about 220 mM, about 230 mM, about 240 mM, about 250 mM, about 260 mM, about 270 mM, about 280 mM, about 290 mM, about 300 mM, about

310 mM, about 320 mM, about 330 mM, about 340 mM, about 350 mM, about 360 mM, about 370 mM, about 380 mM, about 390 mM, about 400 mM, about 410 mM, about 420 mM, about 430 mM, about 440 mM, about 450 mM, about 460 mM, about 470 mM, about 480 mM, about 490 mM or about 500 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 50 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 100 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 150 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 200 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 250 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 300 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 350 mM.

In some embodiments, the pharmaceutical composition comprises sucrose at a concentration of about 400 mM.

In some embodiments, the pharmaceutical composition comprises methionine.

In some embodiments, the pharmaceutical composition comprises methionine at a concentration of from about 0.1 mg/mL to about 5 mg/mL.

In some embodiments, the pharmaceutical composition comprises methionine at a concentration of from about 0.1 mg/mL to about 2.5 mg/mL.

In some embodiments, the pharmaceutical composition comprises methionine at a concentration of from about 1 mg/mL to about 2 mg/mL.

In some embodiments, the pharmaceutical composition comprises methionine at a concentration of about 0.5 mg/mL, about 1 mg/mL, about 1.1 mg/mL, about 1.2 mg/mL, about 1.3 mg/mL, about 1.4 mg/mL, about 1.5 mg/mL, about 1.6 mg/mL, about 1/7 mg/mL, about 1.8 mg/mL, about 1.9 mg/mL, about 2.0 mg/mL, about 2.1 mg/mL, about 2.2 mg/mL, about 2/3 mg/mL, about 2.4 mg/mL, about 2.5 mg/mL, about 2.6 mg/mL,

about 2.7 mg/mL, about 2.8 mg/mL, about 2.9 mg/mL, about 3 mg/mL, about 3.5 mg/mL, about 4 mg/mL, about 4.5 mg/mL or about 5 mg/mL.

In some embodiments, the pharmaceutical composition is at pH 5.0 to 6.0.

In some embodiments, the pharmaceutical composition is at pH 5.3 to 5.8.

In some embodiments, the pharmaceutical composition is at pH 5.5.

In some embodiments, the pharmaceutical composition is at pH 5.6.

The invention also provides a pharmaceutical composition comprising

from about 1 mg/mL to about 180 mg/mL of the anti-CD38 antibody;

from about 50 U/mL to about 5,000 U/mL of the hyaluronidase

from about 5 mM to about 50 mM histidine; and

from about 50 mM to about 400 mM sorbitol.

In some embodiments, the hyaluronidase is rHuPH20.

The invention also provides a pharmaceutical composition comprising

from about 1 mg/mL to about 180 mg/mL of the anti-CD38 antibody;

from about 50 U/mL to about 5,000 U/mL of the hyaluronidase

from about 5 mM to about 50 mM histidine;

from about 50 mM to about 400 mM sorbitol;

from about 0.01% w/v to about 0.1% PS-20; and

from about 0.1 mg/mL to about 2.5 mg/mL methionine.

In some embodiments, the hyaluronidase is rHuPH20.

The invention also provides a pharmaceutical composition comprising

from about 100 mg/mL to about 120 mg/mL of the anti-CD38 antibody;

from about 50 U/mL to about 5,000 U/mL of the hyaluronidase;

about 10 mM histidine; and

from about 100 mM to about 300 mM sorbitol.

In some embodiments, the hyaluronidase is rHuPH20.

In some embodiments, the pharmaceutical composition further comprises from about 0.01% w/v to about 0.04% w/v PS-20.

In some embodiments, the pharmaceutical composition further comprises from about 1 mg/mL to about 2 mg/mL methionine.

In some embodiments, the pharmaceutical composition further comprises from about 100 mM to about 200 mM sucrose.

In some embodiments, the anti-CD38 antibody comprises

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

the VH and the VL of SEQ ID NOs: 4 and 5, respectively; and/or

the heavy chain and the light chain of SEQ ID NOs: 12 and 13, respectively.

In some embodiments, the anti-CD38 antibody comprises

the VH and the VL of SEQ ID NOs: 14 and 15, respectively;

the VH and the VL of SEQ ID NOs: 16 and 17, respectively;

the VH and the VL of SEQ ID NOs: 18 and 19, respectively; or

the VH and the VL of SEQ ID NOs: 20 and 21, respectively;

In some embodiments, the hyaluronidase comprises rHuPH20 (SEQ ID NO: 22)

The invention also provides a pharmaceutical composition comprising

from about 1 mg/mL to about 180 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

from about 50 U/mL to about 5,000 U/mL of the hyaluronidase

from about 5 mM to about 50 mM histidine; and

from about 50 mM to about 400 mM sorbitol.

In some embodiments, the hyaluronidase is rHuPH20.

The invention also provides a pharmaceutical composition comprising

from about 1 mg/mL to about 180 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

from about 50 U/mL to about 5,000 U/mL hyaluronidase

from about 5 mM to about 50 mM histidine;

from about 50 mM to about 400 mM sorbitol;

from about 0.01% w/v to about 0.1% PS-20; and

from about 0.1 mg/mL to about 2.5 mg/mL methionine.

In some embodiments, the hyaluronidase is rHuPH20.

The invention also provides a pharmaceutical composition comprising

from about 100 mg/mL to about 120 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

from about 50 U/mL to about 5,000 U/mL rHuPH20;

about 10 mM histidine;

from about 100 mM to about 300 mM sorbitol;

from about 0.01% w/v to about 0.04% w/v PS-20; and

from about 1 mg/mL to about 2 mg/mL methionine.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 500 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.04% w/v PS-20; and

about 2 mg/mL methionine; at pH about 5.5.

The invention also provides a pharmaceutical composition comprising

about 120 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 2,000 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.04% w/v PS-20; and

about 1 mg/mL methionine; at pH about 5.6.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 500 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol; and

about 2 mg/mL methionine; at pH about 5.5.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 500 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.01% w/v PS-20; and

about 2 mg/mL methionine; at pH about 5.5.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 500 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.02% w/v PS-20; and

about 2 mg/mL methionine; at pH about 5.5.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 500 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.06% w/v PS-20; and

about 2 mg/mL methionine; at pH about 5.5.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 50 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.04% w/v PS-20; and

about 1 mg/mL methionine; at pH about 5.5.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 500 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.04% w/v PS-20; and

about 1 mg/mL methionine; at pH about 5.5.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;
about 2,000 U/mL rHuPH20;
about 10 mM histidine;
about 300 mM sorbitol;
about 0.04% w/v PS-20; and
about 1 mg/mL methionine; at pH about 5.5.

The invention also provides a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;
about 5,000 U/mL rHuPH20;
about 10 mM histidine;
about 300 mM sorbitol;
about 0.04% w/v PS-20; and
about 1 mg/mL methionine; at pH about 5.5.

In some embodiments, the pharmaceutical composition is a fixed combination.

The formulations to be used for *in vivo* administration are generally sterile. Sterility may be readily accomplished, e.g., by filtration through sterile filtration membranes.

The pharmaceutical compositions of the invention may be prepared by known methods. For example, the pharmaceutical compositions may be prepared, e.g., by dissolving, suspending or emulsifying the anti-CD38 antibody in a sterile aqueous medium or an oily medium conventionally used for injections.

Administration

The pharmaceutical compositions of the invention may be administered as a non-fixed combination.

The pharmaceutical compositions of the invention may also be administered as a fixed combination, e.g., as a unit dosage form (or dosage unit form). Fixed combinations may be advantageous for ease of administration and uniformity of dosage.

The invention also provides a unit dosage form, comprising the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount

of from about 1,200 mg to about 5,000 mg and rHuPH20 in an amount of from about 30,000 U to about 75,000 U.

The invention also provides a unit dosage form, comprising the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of from about 1,200 mg to about 4,000 mg and rHuPH20 in an amount of from about 30,000 U to about 75,000 U.

The invention also provides a unit dosage form, comprising the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of from about 1,200 mg to about 2,400 mg and rHuPH20 in an amount of from about 30,000 U to about 45,000 U.

The invention also provides a unit dosage form, comprising the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of from about 1,200 mg to about 1,800 mg and rHuPH20 in an amount of from about 30,000 U to about 45,000 U.

The invention also provides a unit dosage form, comprising

the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of from about 1,200 mg to about 5,000 mg;

rHuPH20 in an amount of from about 30,000 U to about 75,000 U;

histidine at a concentration of from about 5 mM to about 15 mM;

sorbitol at a concentration of from about 100 mM to about 300 mM;

PS-20 at a concentration of from about 0.01% w/v to about 0.04 % w/v; and

methionine at a concentration of from about 1 mg/mL to about 2 mg/mL, at a pH of about 5.5.

The invention also provides a unit dosage form, comprising

the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of about 1,200 mg to about 2,400 mg;

rHuPH20 in an amount of from about 30,000 U to about 45,000 U;

histidine at a concentration of about 10 mM;

sorbitol at a concentration of about 300 mM;

PS-20 at a concentration of about 0.04 % w/v; and

methionine at a concentration of from about 1 mg/mL; at a pH of about 5.5.

The invention also provides a unit dosage form, comprising

the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of about 1,200 mg to about 1,800 mg;

rHuPH20 in an amount of from about 30,000 U to about 45,000 U;

histidine at a concentration of about 10 mM;

sorbitol at a concentration of about 300 mM;

PS-20 at a concentration of about 0.04 % w/v; and

methionine at a concentration of from about 1 mg/mL; at a pH of about 5.5.

The invention also provides a unit dosage form, comprising

the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of from about 1,200 mg to about 1,800 mg;

rHuPH20 in an amount of from about 30,000 U to about 45,000 U;

histidine at a concentration of from about 5 mM to about 15 mM;

sorbitol at a concentration of from about 100 mM to about 300 mM;

PS-20at a concentration of from about 0.01% w/v to about 0.04 % w/v; and

methionine at a concentration of from about 1 mg/mL to about 2 mg/mL, at a pH of about 5.5.

The invention also provides a unit dosage form, comprising

the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of about 1,800 mg;

rHuPH20 in an amount of from about 30,000 U;

histidine at a concentration of about 10 mM;

sorbitol at a concentration of about 300 mM;

PS-20 at a concentration of about 0.04 % w/v; and

methionine at a concentration of from about 1 mg/mL; at a pH of about 5.5.

The invention also provides a unit dosage form, comprising

the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in an amount of about 1,800 mg;
rHuPH20 in an amount of from about 45,000 U;
histidine at a concentration of about 10 mM;
sorbitol at a concentration of about 300 mM;
PS-20 at a concentration of about 0.04 % w/v; and
methionine at a concentration of from about 1 mg/mL; at a pH of about 5.5.

The pharmaceutical composition of the invention may be administered in a total volume of about 80 mL, 90 mL, 100 mL, 110 mL or 120 mL.

The pharmaceutical composition of the invention may be administered in a total volume of about 10 mL, 11 mL, 12 mL, 13 mL, 14 mL, 15 mL, 16 mL, 17 mL, 18 mL, 19 mL, 20 mL, 25 mL, 30 mL, 35 mL, 40 mL, 45 mL, 50 mL, 55 mL, 60 mL, 65 mL, 70 mL, 75 mL, 80 mL, 85 mL, 90 mL, 95 mL, 100 mL, 105 mL, 110 mL, 115 mL or 120 mL.

The pharmaceutical composition of the invention may be administered in a total volume of about 10 mL.

The pharmaceutical composition of the invention may be administered in a total volume of about 15 mL.

The pharmaceutical composition of the invention may be administered in a total volume of about 20 mL.

The total volume of administration may be typically smaller for the fixed combinations when compared to the non-fixed combinations.

The invention also provides a container comprising the pharmaceutical composition of the invention.

The invention also provides a container comprising the unit dosage form of the invention.

The container may be a vial, a cartridge, a syringe, a prefilled syringe or a disposable pen.

The administration of the pharmaceutical compositions of the invention may be repeated after one day, two days, three days, four days, five days, six days, one week, two weeks, three weeks, four weeks, five weeks, six weeks, seven weeks, two months, three months, four months, five months, six months or longer. Repeated courses of treatment are also possible, as is chronic administration. The repeated administration may be at the

same dose or at a different dose. For example, the pharmaceutical compositions of the invention may be administered once weekly for eight weeks, followed by once in two weeks for 16 weeks, followed by once in four weeks.

The pharmaceutical composition of the invention may be administered subcutaneously.

The pharmaceutical composition of the invention may be administered subcutaneously to the abdominal region.

Subcutaneous administration may be accomplished using a device. The device may be a syringe, a prefilled syringe, an auto-injector, either disposable or reusable, a pen injector, a patch injector, a wearable injector or an ambulatory syringe infusion pump with subcutaneous infusion sets.

For non-fixed combinations, 20 mg/mL anti-CD38 antibody in 25 mM sodium acetate, 60 mM sodium chloride, 140 mM D-mannitol, 0.04% polysorbate 20, pH 5.5 may be mixed with 1 mg/mL (75-150 kU/mL) rHuPH20 in 10 mM L-Histidine, 130 mM NaCl, 10 mM L-Methionine, 0.02% polysorbate-80, pH 6.5 prior to administration of the mixture to a subject.

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

Methods of treatment

The invention also provides a method of treating a cancer, comprising administering to a subject in need thereof the pharmaceutical composition of the invention for a time sufficient to treat the cancer.

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

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

In some embodiments, the CD38-positive hematological malignancy is diffuse large B-cell lymphoma (DLBCL).

In some embodiments, the CD38-positive hematological malignancy is non-Hodgkin's lymphoma.

In some embodiments, the CD38-positive hematological malignancy is acute lymphoblastic leukemia (ALL).

In some embodiments, the CD38-positive hematological malignancy is follicular lymphoma (FL).

In some embodiments, the CD38-positive hematological malignancy is Burkitt's lymphoma (BL).

In some embodiments, the CD38-positive hematological malignancy is mantle cell lymphoma (MCL).

In some embodiments, the CD38-positive hematological malignancy is light chain amyloidosis (AL).

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

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

In some embodiments, the cancer is a solid tumor.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising an anti-CD38 antibody and a hyaluronidase subcutaneously for a time sufficient to treat the CD38-positive hematological malignancy, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 and the hyaluronidase rHuPH20 of SEQ ID NO: 22 subcutaneously for a time sufficient to treat the CD38-positive hematological malignancy, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising between about 1,200 mg – 1,800 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and between about 30,000 U – 45,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22 for a time sufficient to treat the CD38-positive hematological malignancy, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising between about 1,200 mg and about 1,800 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 30,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22 for a time sufficient to treat the CD38-positive hematological malignancy, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising between about 1,200 mg and about 1,800 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 45,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22 for a time sufficient to treat the CD38-positive hematological malignancy, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof between about 1,600 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 30,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22 for a time sufficient to treat the CD38-positive hematological malignancy, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof about 1,600 mg of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5, and about 45,000 U of the hyaluronidase rHuPH20 of SEQ ID NO: 22 for a time sufficient to treat the CD38-positive hematological malignancy, wherein the anti-CD38 antibody concentration in the pharmaceutical composition is about 20 mg/mL.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 and the hyaluronidase, wherein the pharmaceutical composition is a non-fixed combination.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising

from about 20 mg/mL to about 120 mg/mL of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in about 25 mM acetic acid, about 60 mM sodium chloride, about 140 mannitol and about 0.04% w/v polysorbate-20 (PS-20); at pH about 5.5; and

about from 30,000 U to about 45,000 U of the hyaluronidase in 10 mM L-histidine, 130 mM NaCl, 10 mM L-methionine, 0.02% Polysorbate-80, pH 6.5.

In some embodiments, the hyaluronidase is rHuPH20.

In some embodiments, the pharmaceutical composition is a non-fixed combination.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising

about 20 mg/mL of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in about 25 mM acetic acid, about 60 mM sodium chloride, about 140 mannitol and about 0.04% w/v polysorbate-20 (PS-20); at pH about 5.5; and

about 30,000 U of the hyaluronidase in 10 mM L-histidine, 130 mM NaCl, 10 mM L-methionine, 0.02% Polysorbate-80, pH 6.5.

In some embodiments, the hyaluronidase is rHuPH20.

In some embodiments, the pharmaceutical composition is a non-fixed combination.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising

about 20 mg/mL of the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 in about 25 mM acetic acid, about 60 mM sodium chloride, about 140 mannitol and about 0.04% w/v polysorbate-20 (PS-20); at pH about 5.5; and

about 45,000 U of the hyaluronidase in 10 mM L-histidine, 130 mM NaCl, 10 mM L-methionine, 0.02% Polysorbate-80, pH 6.5.

In some embodiments, the hyaluronidase is rHuPH20.

In some embodiments, the pharmaceutical composition is a non-fixed combination.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising the anti-CD38 antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 and the hyaluronidase, wherein the pharmaceutical composition is a fixed combination.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising

from about 1 mg/mL to about 180 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

from about 50 U/mL to about 5,000 U/mL of the hyaluronidase

from about 5 mM to about 50 mM histidine; and

from about 50 mM to about 400 mM sorbitol.

In some embodiments, the hyaluronidase is rHuPH20.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising

from about 1 mg/mL to about 180 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

from about 50 U/mL to about 5,000 U/mL of the hyaluronidase

from about 5 mM to about 50 mM histidine;

from about 50 mM to about 400 mM sorbitol;

from about 0.01% w/v to about 0.1% PS-20; and

from about 0.1 mg/mL to about 2.5 mg/mL methionine.

In some embodiments, the hyaluronidase is rHuPH20.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising

from about 100 mg/mL to about 120 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

from about 50 U/mL to about 5,000 U/mL of the hyaluronidase;

about 10 mM histidine;

from about 100 mM to about 300 mM sorbitol;

from about 0.01% w/v to about 0.04% w/v PS-20; and

from about 1 mg/mL to about 2 mg/mL methionine.

In some embodiments, the hyaluronidase is rHuPH20.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising

about 100 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 500 U/mL of the hyaluronidase;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.04% w/v PS-20; and

about 2 mg/mL methionine at pH about 5.5.

In some embodiments, the hyaluronidase is rHuPH20.

The invention also provides a method of treating a CD38-positive hematological malignancy, comprising administering to a subject in need thereof a pharmaceutical composition comprising

about 120 mg/mL of the anti-CD38 antibody comprising the VH and the VL of SEQ ID NOs: 4 and 5, respectively;

about 2,000 U/mL rHuPH20;

about 10 mM histidine;

about 300 mM sorbitol;

about 0.04% w/v PS-20; and

about 1 mg/mL methionine; at pH about 5.6.

In some embodiments, the hyaluronidase is rHuPH20.

The anti-CD38 antibodies in the pharmaceutical compositions of the invention may induce killing ofCD38-expressing tumor cells by antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement dependent cytotoxicity (CDC), apoptosis, or modulation of CD38 enzymatic activity. The anti-CD38 antibodies in the pharmaceutical compositions of the invention may also mediate anti-tumor efficacy by their immunomodulatory effects by inducing $CD4^+$ and $CD8^+$ T cell proliferation, and/or by relieving inhibition of inflammatory responses mediated by myeloid-derived suppressor cells (MDSCs) and regulatory T cells (Tregs).

"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 antibody that specifically binds CD38, 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 Tregs or MDSCs 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 target 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.

"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 Tregs or MDSCs 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.

"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.

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 embodiments, the anti-CD38 antibody comprises a substitution in the antibody Fc.

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

Substitutions in the Fc and reduced fucose content may enhance the ADCC activity of the antibody that specifically binds CD38.

"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 may be labeled with a fluorophore, separated and identified by various complementary techniques which allow: fine characterization of the glycan structures by matrix-assisted laser desorption ionization (MALDI) mass spectrometry by comparison of the experimental masses with the theoretical masses, determination of the degree of sialylation by ion exchange HPLC (GlycoSep C), separation and quantification of the 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 herein refers to antibodies with fucose content of about 0% - 15%.

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

In 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.

Antibodies that are substantially identical to the antibody comprising the VH of SEQ ID NO: 4 and the VL of SEQ ID NO: 5 may be used in the methods of the invention. The term "substantially identical" as used herein means that the two antibody VH or VL 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 may 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, their ability to induce ADCC, ADCP or apoptosis, or modulate CD38 enzymatic activity *in vitro* using methods described herein.

In some embodiments, the anti-CD38 antibody may bind human CD38 with a range of affinities (K_D). In one embodiment according to the invention, and in some embodiments of each and every one of the numbered embodiments listed below, the anti-CD38 antibody binds to CD38 with high affinity, for example, with a K_D equal to or less than about 10^{-7} M, such as but not limited to, 1-9.9 (or any range or value therein, such as 1, 2, 3, 4, 5, 6, 7, 8, or 9) x 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, 10^{-12} M, 10^{-13} M, 10^{-14} M, 10^{-15} M or any range or value therein, as determined by surface plasmon resonance or the Kinexa method, as practiced by those of skill in the art. One exemplary affinity is equal to or less than $1x10^{-8}$ M. Another exemplary affinity is equal to or less than $1x10^{-9}$ M.

In some embodiments, the anti-CD38 antibody is a bispecific antibody. The VL and/or the VH regions of the existing anti-CD38 antibodies or the VL and VH regions identified *de novo* as described herein may be engineered into bispecific full length antibodies. Such bispecific antibodies may be made by modulating the CH3 interactions between the monospecific antibody heavy chains to form bispecific antibodies using technologies such as those described in U.S. Pat. No. 7,695,936; Intl. Pat. Publ. No. WO04/111233; U.S. Pat. Publ. No. US2010/0015133; U.S. Pat. Publ. No.

US2007/0287170; Intl. 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; Intl. Pat. Publ. No. WO2011/131746; Int. Pat. Publ. No. WO2011/143545; or U.S. Pat. Publ. No. US2012/0149876. Additional bispecific structures into which the VL and/or the VH regions of the antibodies of the invention may be incorporated are for example Dual Variable Domain Immunoglobulins (Inlt. 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).

For example, bispecific antibodies 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 parental 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.

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

Combination therapies

The pharmaceutical compositions of the invention may be administered in combination with a second therapeutic agent, or combinations thereof.

The second therapeutic agent may be melphalan, mechlorethamine, thioepa, chlorambucil, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, dacarbazine (DTIC), procarbazine, mitomycin C, cisplatin and other platinum derivatives, such as carboplatin, thalidomide or a thalidomide analog, lenalidomide or CC4047, a proteasome inhibitor, such as bortezomib or vinca alkaloid, such as vincristine or an anthracycline, such as doxorubicin.

In some embodiments, the second therapeutic agent is a proteasome inhibitor.

In some embodiments, the proteasome inhibitor is bortezomib, carfilzomib or ixazomib.

In some embodiments, the second therapeutic agent is an alkylating agent.

In some embodiments, the alkylating agent is busulfan, cyclophosphamide, bendamustine, chlorambucli, carboplatin, cisplatin, temozolomide, melphalan, busulfan, bendamustine, carmustine, lomustine, dacarbazine, oxaliplatin, ifosfamide, mechlorethamine, thiotepa, trabectedin or streptozocin.

In some embodiments, the second therapeutic agent is a glutamic acid derivative.

In some embodiments, the glutamic acid derivative is Revlimid® (lenalidomide), thalidomide or Pomalyst® (pomalidomide).

In some embodiments, the subject is further administered a corticosteroid.

In some embodiments, the corticosteroid is dexamethasone or predisone.

The second therapeutic agent or combinations thereof are typically administered at dosages recommended for the agent.

The pharmaceutical composition of the invention may be administered simultaneously or sequentially with the second thereapeutic agent or combinations thereof.

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.

Example 1.Subcutaneous delivery of 2% human immunoglobulin G (IgG) with recombinant human hyaluronidase PH20 (rHuPH20) in the miniature swine model

Summary

The miniature pig is a preclinical model that is suitable for evaluating subcutaneous (SC) administration conditions of biotherapeutics due to its anatomical similarity to human skin and clinical translatability (Mahj *et al.,* Exp Toxicol Path 57: 341-5,2006). The objective of this study was to assess and evaluate conditions for a 100 mL administration of a 20 mg/mL human IgG solution containing 200, 500, or 800 U/mL of rHuPH20 at two different flow rates (2 and 4 mL/min). Endpoints included quantitative infusion pressure measurements as well as qualitative assessments of the local infusion site, such as swelling size and firmness.

Yucatan miniature pigs were subcutaneously infused with 100 mL of a solution containing 20 mg/mL immunoglobulin G (IgG) with 200, 500, or 800 U/mL of rHuPH20 at a flow rate of 2 or 4 mL/min. Real-time in-line pressures were measured during infusions. Following the completion of infusions, local infusion sites were measured for visible swelling volume and area if present, and qualitative scoring of the infusion site for presence and severity of erythema, size and firmness of the swelling/bleb, and gross observations. Infusion pressures were low overall and ranged from ~ 40 to 60 mmHg (~ 1 PSI) for both flow rates.

There were no statistical differences in pressure between the various concentrations of rHuPH20 and the two different flow rates, with overall pressure being slightly lower at the 2 mL/min flow rate as expected.

The unexpected finding was the number of infusions (10 of 12) that had visible and measurable swelling at the infusion site for the lower 2 mL/min flow rate. This observation was observed at all three concentrations of rHuPH20. In contrast, only 3 of 12 infusions at the higher 4 mL/min flow rate resulted in visible and measurable local swelling. Again this was observed at each concentration of rHuPH20.

In all cases where local swelling was visible, the swelling subsided within an hour. Additionally, local swelling at the infusion sites was generally soft to the touch and not indurated, as indicated by the swelling/induration index (mean score < 2). The prevalence of erythema was more frequent with infusions at the 2 mL/min flow rate; however, overall

the severity of the erythema was mild and completely subsided by the following day. No other gross observations of the infusion sites were noted in the study.

Three different concentrations of rHuPH20 (200, 500, or 800 U/mL) were evaluated in this study, with no statistical difference between the concentrations based on the endpoints of the study. Overall, the higher 4 mL/min flow rate resulted in lower frequency of erythema, visible swelling, and local infusion site firmness than the 2 mL/min flow rate.

Test articles and methods

Test Articles

Materials in Formulation Buffer:

- 25mM sodium acetate (Spectrum; PN#S0104; Lot#1DI0271)
- 60 mM sodium chloride (Spectrum; PN#S0155; Lot#1CE0421)
- 140 mM D-mannitol (Spectrum; PN#MA165; Lot#1EB0316)
- 0.04% polysorbate 20 (JT Baker; PN#4116-04; Lot#0000017659)
- pH 5.5 (glacial acetic acid to pH; Fisher Scientific; PN#A491-212; Lot#080972)

Materials in Drug Substance:

- Human Gamma Globulin (BioMed Supply; PN#HGG-1005; Lot#BMS31309013)
- rHuPH20 [(Manufactured by Cook Pharmica for Halozyme; Halozyme Lot#462-
- 021B (revialed, Cook Lot#104-001-HSTFIL-9054)]

Formulation

20 mg/mL IgG was co-mixed with 200, 500, or 800 U/mL of rHuPH20 4 days prior to study onset. The solutions were aliquoted into individual glass bottles, sealed with a stopper, and crimp capped. All solutions were stored at 2-8°C until start of study, but were allowed to acclimate to room temperature prior to infusions. Additionally, a sample was taken from each formulation for rHuPH20 enzymatic activity testing. Results of the enzymatic activity assay confirmed that all dosing solutions were within 10% of the target concentration (data not shown).

Animal Description

- Species: Pig (*Sus scrofa domestica*)

- Strain: Yucatan Miniature
- Sex: Female
- Age: > 3 months
- Weight: ~ 12 kg
- Quantity: 12
- Source: S&S Farms (Ramona, CA)

Husbandry

Animals were housed in steel pens with automatic water provided *ad libitum*. Animals were fed twice daily (AM and PM), except on study day (PM only). Animal body weights were taken and recorded from day of delivery to one day post-completion of the study to assess animal health. All animals maintained body weights during this period (data not shown). The room environment was set to maintain a temperature of ~17-27°C and a relative humidity of 40-70%, with a 12 hour light/12 hour dark time cycle. Animals were allowed to acclimate to the facility for 7 days prior to study onset.

Test Materials

• High pressure syringe pumps (KD Scientific; Holliston, MA)
• 23 ga x ¾ inch winged infusion needle set with 12 inch tubing (Terumo Medical Corporation; Somerset, NJ)
• 140-cc Luer-lock syringe (Covidien; Mansfield, MA)
• Extension set 7 inch (B/Braun; Bethlehem, PA)
• PowerLab 4/30 (AD Instruments; Colorado Springs, CO)
• Deltran-1 disposable pressure transducer (Utah Medical Products; Midvale, UT)
• Digital caliper (Preisser Messtechnik; Gammertingen, Germany)
• Isoflurane (Minrad International Company, Orchard Park, NY)
• Isoflurane vaporizer (VetEquip; Pleasanton, CA)

Experimental design

The experimental design is summarized in the Description of Cohorts (**Table 1**) and Description of Infusions per Animal (**Table 2**). In brief, 100 mL of a solution containing 20 mg/mL IgG co-mixed with 200, 500, or 800 U/mL of rHuPH20 was

administered into the abdominal region of anesthetized Yucatan mini-pigs at a flow rate of 2 or 4 mL/min. The endpoints of the study included infusion pressure measurements using an in-line pressure transducer, local post-infusion swelling (bleb) volume and area (if possible), and qualitative assessment of the infusion site, including photographs.

Table 1.

Cohort	Test Article	Flow Rate (mL/min)
1	IgG + 200 U/mL rHuPH20	2 mL/min
2	IgG + 500 U/mL rHuPH20	
3	IgG + 800 U/mL rHuPH20	
4	IgG + 200 U/mL rHuPH20	4 mL/min
5	IgG + 500 U/mL rHuPH20	
6	IgG + 800 U/mL rHuPH20	

Table 2.

Animal ID	Flow rate (mL/min)	Left side infusion	Right side infusion
1	2	IgG + 200 U/mL rHuPH20	IgG + 500 U/mL rHuPH20
2		IgG + 200 U/mL rHuPH20	IgG + 800 U/mL rHuPH20
3		IgG + 500 U/mL rHuPH20	IgG + 200 U/mL rHuPH20
4		IgG + 500 U/mL rHuPH20	IgG + 800 U/mL rHuPH20
5		IgG + 800 U/mL rHuPH20	IgG + 200 U/mL rHuPH20
6		IgG + 800 U/mL rHuPH20	IgG + 500 U/mL rHuPH20
7	4	IgG + 200 U/mL rHuPH20	IgG + 500 U/mL rHuPH20
8		IgG + 200 U/mL rHuPH20	IgG + 800 U/mL rHuPH20
9		IgG + 500 U/mL rHuPH20	IgG + 200 U/mL rHuPH20
10		IgG + 500 U/mL rHuPH20	IgG + 800 U/mL rHuPH20
11		IgG + 800 U/mL rHuPH20	IgG + 200 U/mL rHuPH20
12		IgG + 800 U/mL rHuPH20	IgG + 500 U/mL rHuPH20
n = 4 infusions per cohort per flow rate			

Each animal received 2 different simultaneous infusions (one on each contra-lateral site) rHuPH20 concentrations: 200 U/mL = 20,000 total U; 500 U/mL = 50,000 total U; 800 U/mL = 80,000 total U Infusion volume 100 mL Endpoints: o In-line (infusion) pressure o Measurement of swelling/bleb volume and area o Qualitative assessment of erythema and induration including photographs

Study Procedure

Prior to start of study, animals were assessed for general health and body weights were collected. On the day of study, animals were anesthetized with isoflurane gas and placed in dorsal recumbence on a heated surgical table, and were maintained under isoflurane gas for the entire duration of the procedure. The abdominal region was cleaned with isopropanol and wiped dry with clean gauze. Infusion sites were located on the left and right abdominal regions, ~ 3-4 cm towards the midline starting from the cranial end of the inguinal fold and then ~ 6 cm cranial. The infusion sites were marked with a permanent marker and then photographed. Test articles were acclimated to room temperature prior to infusions. Test articles were drawn into a 140 cc syringe (> 100 mL to account for volume needed to prime the line). A pressure transducer was attached to the syringe. A line extension set with an attached 23 ga x ¾ inch winged infusion needle was then attached to the transducer. The infusion hardware was then primed to the needle tip. The syringe was loaded into the syringe pump. This process was done in duplicate, with each syringe containing a different test article. The needles were placed subcutaneously into the marked left and right abdominal infusion sites of the animal. The in-line pressure transducer was zeroed. In-line pressure recordings were started and then the two syringe pumps were started simultaneously to infuse 100 mL of test articles at a flow rate of 2 or 4 mL/min. Upon completion of infusions, in-line pressure data collection were stopped, needles removed, and the needle insertion hole sealed with VetBond liquid adhesive to prevent any leakage. Local infusion site swelling/bleb area and volume were measured using a digital caliper. Local infusion sites were also qualitatively assessed for

appearance (erythema), swelling/bleb size, and firmness (induration) using a 5 point scoring system (**Table 3, Table 4, and Table 5**, respectively). Finally, photographs were taken of the infusion sites.

Table 3.

Scale	Description
0	No erythema
1	Very slight erythema (barely perceptible)
2	Well defined erythema
3	Moderate to severe erythema
4	Severe erythema (beet redness) to slight eschar formation

Table 4.

Scale	Description
0	No swelling
1	Very slight swelling
2	Slight swelling
3	Moderate swelling
4	Severe swelling

Table 5.

Scale	Description
0	No perceptible difference in firmness after injection
1	Very slightly firm (barely perceptible)
2	Mildly firm
3	Moderately firm
4	Very firm

Calculations and statistical methods

Assessment of Infusion Pressure:

Infusion pressures, as measured via an in-line transducer, were recorded using LabChart 7, and the mean pressure over the entire infusion period was calculated.

Assessment of Local Swelling Volume and Area:

Volume and area of post-infusion swelling were measured using a digital caliper and manually recorded. Measurements were recorded as length, width, and height. The formula for an ellipsoid was used to calculate volume. Volume = $4/3\pi ABC$, where A = radius of length, B = radius of width, C = radius of height. A simple formula of length x width was used to calculate the area.

Assessment of Local Infusion Sites:

Local infusion sites were independently assessed by three separate evaluators following the completion of the infusion. Each evaluator assessed the skin at each infusion site for the presence of erythema, size of local swelling, and firmness. A score on a 0 to 4 grading scale was used to evaluate the three areas of assessment, with a score of 0 representing no effect and 4 being severe. Additionally, erythema and swelling scores were used to calculate a primary irritation index (PII) using the formula PII = mean [(Σ of erythema grade + Σ of swelling grade)÷ 2]. Furthermore, the swelling and firmness scores were used to calculate a swelling/induration index (SII) using the formula SII = mean [(Σ of swelling grade + Σ of hardness grade) ÷ 2]. A SII score of ≤ 2 was not considered to be indurated.

Statistical Analyses:

Statistical comparisons between cohorts were performed using an one-way analysis of variance (ANOVA) with a Tukey's multiple comparisons test for continuous variables, and a nonparameteric Krusal-Wallis test with a Dunn's multiple comparisons test for categorical variables. Statistical significance was determined to be $p < 0.05$.

RESULTS

Assessment of Infusion Pressure:

100 mL of 20 mg/mL IgG co-mixed with 200, 500, or 800 U/mL of rHuPH20 was administered into the abdominal region of Yucatan mini-pigs at a flow rate of 2 or 4 mL/min. Infusions at a flow rate of 2 mL/min resulted in mean infusion pressures of 40.5 ± 0.1, 40.0 ± 0.1, and 37.1 ±0.1 mmHg ± SEM for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. Infusions at a flow rate of 4 mL/min resulted in mean

pressures of 49.9 ± 0.1, 55.5 ±0.1, and 61.9 ± 0.2 mmHg ± SEM for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. The infusion pressures were not statistically different between the various concentrations of rHuPH20 for each flow rate and were not statistically different between the two flow rates.

Assessment of Local Swelling Volume and Area:

After completion of each infusion, the local infusion site swelling was marked if visible and measured using a digital caliper. For infusions at a flow rate of 2 mL/min resulted in a mean swelling volume of 36.6 ± 14.4, 19.5 ± 6.5, and 31.4 ± 6.0 cm3 ± SEM for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively, of which 10 of 12 infusions were visible and measurable. On the contrary, the swelling volume was only detected in 3 of 12 infusions at a flow rate of 4 mL/min, one at each rHuPH20 concentration. The local swelling volumes were not statistically different between the various concentrations of rHuPH20 for each flow rate and were not statistically different between the two flow rates.

In addition to volume calculations, the local area of post-infusion visible swelling was measured. For infusions at a flow rate of 2 mL/min resulted in a mean swelling area of 78.2 ± 26.4, 59.7 ±20.0, and 94.9 ± 9.7 cm2 ± SEM for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. For infusions at a flow rate of 4 mL/min resulted in measurable swelling areas in only 3 of 12 infusions, one at each rHuPH20 concentration. The local swelling areas were not statistically different between the various concentrations of rHuPH20 for each flow rate and were not statistically different between the two flow rates.

Assessment of Local Infusion Sites:

Upon completion of infusions, the local infusion sites were qualitatively scored, by three separate evaluators, for presence and severity of erythema, visible size of swelling, physical firmness of the skin, primary irritation index (PII) which incorporates the erythema and swelling scores, and swelling/induration index (SII) which incorporates the swelling and firmness scores to determine if induration was present.

The presence and severity of erythema was evaluated. For infusions at a flow rate of 2 mL/min resulted in a mean erythema score of (± SEM) of 0.8 ± 0.2, 0.4 ± 0.1, and 1.0 ± 0.3 for IgG commixed with 200, 500, and 800 U/mL of rHuPH20, respectively.

Infusions at a flow rate of 4 mL/min resulted in a mean erythema score (± SEM) of 0.3 ± 0.1, 0.3 ± 0.1. and 0.2 ±0.1 for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. The local erythema scores were not statistically different between the various concentrations of rHuPH20 for each flow rate and were not statistically different between the two flow rates. The size of visible local swelling was evaluated, and for infusions at a flow rate of 2 mL/min resulted in a mean swelling score (± SEM) of 1.9 ± 0.4, 1.4 ± 0.3, and 2.0 ± 0.2 for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. Infusions at a flow rate of 4 mL/min had fewer visible swelling with a mean erythema score (± SEM) of 0.6 ± 0.3, 0.9 ±0.4, and 0.9 ± 0.4 for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. The local swelling scores were not statistically different between the various concentrations of rHuPH20 for each flow rate and was not statistically different between the two flow rates, except for IgG + 800 U/mL of rHuPH20 at 2 mL/min flow rate versus IgG +200 U/mL of rHuPH20 at 4 mL/min flow rate ($p < 0.05$). The physical firmness of the skin at the local infusion site was evaluated, and for infusions at a flow rate of 2 mL/min resulted in a mean firmness score (± SEM) of 1.5 ± 0.3, 1.0 ± 0.2, and 1.4± 0.2 for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. Infusions at a flow rate of 4 mL/min had less local infusion site firmness with a mean firmness score (± SEM) of 0.5 ± 0.3, 0.7 ± 0.2, and 0.7 ± 0.3 for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. The local infusion site firmness scores were not statistically different between the various concentrations of rHuPH20 for each flow rate and were not statistically different between the two flow rates. The primary irritation index was calculated based on the erythema and swelling scores. Infusions at 2 mL/min had a mean PII score (± SEM) of 1.4 ± 0.3, 0.9 ± 0.2, and 1.5 ± 0.2 for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. Infusions at a flow rate of 4 mL/min had lower PII scores with a mean score (± SEM) of 0.4 ± 0.2, 0.6 ± 0.3, and 0.5 ± 0.3 for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. The PII scores were not statistically different between the various concentrations of rHuPH20 for each flow rate and was not statistically different between the two flow rates, except for IgG + 800 U/mL of rHuPH20 at 2 mL/min flow rate versus IgG + 200 U/mL of rHuPH20 at 4 mL/min flow rate ($p < 0.05$). The swelling/induration index was calculated based on the swelling and firmness scores. Infusions at 2 mL/min had a mean SII score (± SEM) of 1.7 ± 0.3, 1.2 ± 0.2, and 1.7 ± 0.2 for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. Infusions at a flow rate of 4 mL/min

had lower SII scores with a mean score of 0.6 ± 0.3, 0.8 ± 0.3, and 0.8 ±0.3 (± SEM) for IgG co-mixed with 200, 500, and 800 U/mL of rHuPH20, respectively. The SII scores were not statistically different between the various concentrations of rHuPH20 for each flow rate and was not statistically different between the two flow rates. Based on the mean SII values (score of ≤ 2), the local infusions sites were considered not to be indurated. Lastly, photographs were taken prior to and after completion of each infusion.

Example 2. An Open-label, Multicenter, Dose Escalation Phase 1b Study to Assess the Safety and Pharmacokinetics of Subcutaneous Delivery of Daratumumab with the Addition of Recombinant Human Hyaluronidase (rHuPH20) for the Treatment of Subjects with Relapsed or Refractory Multiple Myeloma

The purpose of the study is to evaluate the safety, pharmacokinetics and antitumor activity of subcutaneous (SC) or intravenous (IV) delivery of daratumumab to participants with relapsed or refractory multiple myeloma. This is an open-label multicenter, 2-part, Phase 1b dose escalation/expansion study to evaluate the safety, pharmacokinetics and antitumor activity of SC or IV delivery of daratumumab to participant with relapsed or refractory multiple myeloma. Up to approximately 48 participants in part 1 and 80 participants in part 2 will be enrolled. The Part 1 dose escalation phase is designed to determine the recommended Phase 2 dose (RP2D) based on safety and pharmacokinetic (PK) data of daratumumab. Each part of the study will have 3 phases: a Screening Phase, an open label treatment phase and a post-treatment phase (from the final dose of study drug until post-treatment week 8). In Part 1, participants will be assigned to sequential cohorts of approximately 8 participants each cohort. Participants will be dosed with DARA PH20 (Daratumumab with the Addition of Recombinant Human Hyaluronidase [rHuPH20]) by SC infusion once weekly in Cycles 1 (each cycle 28 days) and 2, every 2 weeks in Cycles 3–6, and every 4 weeks in subsequent cycles of each cohort. After the last participant in each cohort completes Cycle 3 day 1, the Safety Evaluation Team (SET) will evaluate the safety and pharmacokinetic data according to protocol-defined criteria and make the decision whether to escalate the dose in a new cohort. The SET will review all safety and PK data from Part 1 to determine the RP2D before initiation of Part 2. In Part 2, participants will be randomized 1:1 to receive recommended Phase 2 dose of

DARA PH20 or IV delivery of 1200 mg DARA. Safety, pharmacokinetics, and antitumor activity of SC and IV delivery of daratumumab will be evaluated. Participant's safety will be monitored throughout the study.

Primary Outcome Measures:

Serum Trough Concentrations (Ctrough) of Daratumumab (Time Frame: Up to part 2 cycle 3 (each cycle 28 days) Day 1). Ctrough: the concentration prior to study drug administration.

Part 1 and 2: Number of Participants with Adverse Events (AEs) and Serious AEs (Time Frame: Screening up to follow-up (30 days after last dose administration)

An adverse event (AE) is any untoward medical occurrence in a participant who received study drug without regard to possibility of causal relationship. A serious adverse event (SAE) is an AE resulting in any of the following outcomes or deemed significant for any other reason: death; initial or prolonged inpatient hospitalization; life-threatening experience (immediate risk of dying); persistent or significant disability/incapacity; congenital anomaly.

Secondary Outcome Measures:

- Part 1 and 2: Serum Concentration of Daratumumab and Recombinant Human Hyaluronidase (rHuPH20) (Plasma) Antibodies (Time Frame: Approximately 2 years). Serum levels of antibodies to Daratumumab and rHuPH20 for evaluation of potential immunogenicity.
- Part 1 and 2: Percentage of Participants with Complete Response (CR) (Time Frame: Approximately 2 years). CR is Defined as the proportion of Participants achieving CR (including sCR) according to the International Myeloma Working Group (IMWG) criteria.
- Part 1 and 2: Percentage of Participants With Overall Response Rate (ORR) (Time Frame: Approximately 2 years). Overall response rate is defined as the percentage of participants who achieve complete response or partial response according to the International Myeloma Working Group criteria, during or after study treatment.

- Part 1 and 2: Duration of Response (DR) (Time Frame: Approximately 2 years). The DR is time from date of initial documentation of response (CR or PR) to date of first documented PD, as defined by IMWG criteria.
- Part 1 and 2: Time to Response (Time Frame: Approximately 2 years). Time to response is defined as the time from the date of first dose of study treatment to the date of the first documentation of observed response (CR or PR).

Table 6 shows the study design. **Table 7** shows the interventions.

Table 6.

Arm Number or Name	Type	Description
Part 1: Cohort 1	Experimental	Participants will receive DARAPH20, 1200 mg (daratumumab 1200 milligram (mg) with Recombinant Human Hyaluronidase [rHuPH20] 30,000 U) by Subcutaneous (SC) infusion once weekly in Cycles 1 (each cycle is 28 days) and 2, every 2 weeks in Cycles 3-6, and then every 4 weeks in subsequent cycles until disease progression.
Part 1: Cohort 2	Experimental	Participants will receive DARAPH20, 1800 mg (daratumumab 1800 milligram (mg) with Recombinant Human Hyaluronidase [rHuPH20] 45,000 U) by SC infusion once weekly in Cycles 1 and 2, every 2 weeks in Cycles 3-6, and then every 4 weeks in subsequent cycles until disease progression.
Part 1: Cohort 3	Experimental	Participants will receive DARAPH20 at a dose which will be decide by Study Evaluation Team (SET) once weekly by SC infusion in Cycles 1 and 2, every 2 weeks in Cycles 3-6, and then every 4

		weeks in subsequent cycles until disease progression. Also up to three additional optional cohorts (Cohorts 3b, 3c, and 3d) may be enrolled to repeat a dose level of daratumumab
Part 2: Cohort 4	Experimental	DARA-PH20 SC infusion at the RP2D (recommended Phase 2 dose) that is identified in Part 1 will be administered to participants by SC infusion once weekly in Cycles 1 and 2, every 2 weeks in Cycles 3-6, and then every 4 weeks in subsequent cycles until disease progression.
Part 2: Cohort 5	Experimental	Daratumumab 1200 mg will be administered to participants by intravenous (IV) once weekly in Cycles 1 and 2, every 2 weeks in Cycles 3-6, and then every 4 weeks in subsequent cycles until disease progression.

Table 7.

Intervention Name	Type	Associated Arms	Description
Daratumumab Subcutaneous (SC) infusion	Drug	Part 1: Cohort 1 Part 1: Cohort 2 Part 1: Cohort 3 Part 2: Cohort 4	Participants will receive Daratumumab sc infusion once weekly in Cycles 1 (each cycle is 28 days) and 2, every 2 weeks in Cycles 3–6, and every 4 weeks in subsequent cycles.
Recombinant Human		Part 1: Cohort 1 Part 1: Cohort 2 Part 1:	Participants will receive Recombinant Human

Hyaluronidase [rHuPH20]) SC infusion		Cohort 3 Part 2: Cohort 4	Hyaluronidase [rHuPH20]) along with Daratumumab by SC infusion once weekly in Cycles 1 (each cycle is 28 days) and 2, every 2 weeks in Cycles 3–6, and every 4 weeks in
Daratumumab Intravenous (IV) infusion		Part 2: Cohort 5	Participants will receive Daratumumab 1200 mg IV infusion once weekly in Cycles 1 (each cycle 28 days) and 2, every 2 weeks in Cycles 3–6, and every 4 weeks in subsequent cycles.

Eligibility

Participants proven to have symptomatic (having symptoms) multiple myeloma (MM) according the International Myeloma Working Group (IMWG) diagnostic Criteria:

-Measurable disease as defined by any of the following: (a) immunoglobulin (Ig) G myeloma (serum monoclonalparaprotein [M-protein] level >=1.0 gram/deciliter [g/dL] or urine M-protein level greater than or equal to (>=) 200 milligram[mg]/24 hours[hrs]; or (b) IgA, IgD, or IgE multiple myeloma (serum M-protein level >= 0.5 g/dL or urine Mprotein level >= 200 mg/24 hrs); or (c) light chain multiple myeloma (serum immunoglobulin free light chain >=10 mg/dL and abnormal serum immunoglobulin kappa lambda free light chain ratio)

- Participant must have an Eastern Cooperative Oncology Group (ECOG) performance status score of 0, 1, or 2

- Pretreatment clinical laboratory values must meet protocoldefined parameters during the Screening phase
- Man, who is sexually active with a woman of child-bearing potential and has not had a vasectomy, must agree to use an adequate contraception method as deemed appropriate by the Investigator, and must also agree to not donate sperm during the study and 4 months after last dose of daratumumab

Exclusion Criteria:

- Participant has received daratumumab or other anti-cluster of differentiation 38 (anti-CD38) therapies previously
- Participant has received anti-myeloma treatment within 2 weeks before Cycle 1 Day 1
- Participant has previously received an allogenic stem cell transplant; or participant has received autologous stem cell transplantation (ASCT) within 12 weeks before Cycle 1 Day1
-Participant has a history of malignancy (other than multiple myeloma) within 5 years before Cycle 1 Day 1 (exceptions are squamous and basal cell carcinomas of the skin and carcinoma in situ of the cervix, or malignancy that in the opinion of the Investigator, with concurrence with the Sponsor's medical monitor, is considered cured with minimal risk of recurrence)
- Participant is exhibiting clinical signs of meningeal involvement of multiple myeloma

Gender: Both

Age limit: 18 years

Accepts healthy volunteers: No

Interim readout of Part 1 (data cutoff 21JUL2016 for safety/demographics/disease history and 28JUL2016 for the efficacy data)

Methods

Patients had RRMM with ≥2 prior lines of therapy including a proteasome inhibitor (PI) and an immunomodulatory drug (IMiD). Part 1 of the 2 part study enrolled sequential cohorts at 1200 mg and 1800 mg DARA dose levels to determine the recommended SC dose for Part 2. DARA-PH20 was administered in 4-week treatment cycles: QW for 8 weeks, Q2W for 16 weeks, and Q4W thereafter. DARA-PH20 was infused in 1200 mg doses in 60 mL over 20 min or 1800 mg in 90 mL over 30 min, via a

syringe pump at rotating sites on the abdomen. Pre- and/or post-infusion medications included paracetamol, diphenhydramine, montelukast, and methylprednisolone. In part 2, pts will be randomized 1:1 to receive the recommended phase 2 dose (RP2D) of SC DARA-PH20 or IV DARA (16 mg/kg). The RP2D of DARA-PH20 will be selected based on a cumulative review of the pharmacokinetic and safety data obtained from part 1 and should achieve a maximum serum Ctrough during weekly dosing that is similar to or higher than that observed for the approved 16 mg/kg IV dose. Primary endpoints were Ctrough of DARA up to Cycle 3 Day 1 and safety. Secondary endpoints included overall response rate (ORR).

Results

To date, 41 pts were treated in part 1 with SC DARA-PH20 at the 1200 mg (n=8) and 1800 mg (n=33) dose levels. Infusion related reactions (IRRs) were reported in 9/41 pts (22%) and were mostly grade 1/2 in severity including chills, fever, rigors, vomiting, itching, edema of the tongue, non-cardiac chest pain and wheezing. One pt developed grade 3 dyspnea and 1 pt required hospitalization due to fever and chills (both grade 2) after the first infusion. All IRRs developed during or within 6 hours of the first SC infusion and were controlled with antihistamine, corticosteroid, antiemetic, or bronchodilator treatment. No IRRs were reported with subsequent infusions. Overall, the adverse event profile of DARA-PH20 was consistent with that of IV DARA. Grade 3 or higher drug-related adverse events were reported in 5/41 (12%) pts including fatigue (2 pts), influenza, hypertension, dyspnea, and tumor lysis syndrome. SC administration of DARA-PH20 was well tolerated at the abdominal wall injection site with 3/41 (7%) pts reporting grade 1 erythema, induration, or burning sensation. Analysis showed a higher max Ctrough in the 1800 mg cohort in comparison to the max Ctrough achieved following IV DARA (16 mg/kg).

In the 1200 mg cohort of 8 pts (median of 5 lines of prior therapy [range 2-10]; prior ASCT, 63%; PI refractory only, 0%; IMiD refractory only, 13%; double refractory to PI and IMiD, 63%) a 25% ORR was observed including 2 partial responses (PR). Median time to response was 14 (range 8-20) weeks. Among 17 response evaluable pts in the 1800 mg cohort with cycle 3 day 1 assessments (median of 4 prior lines of therapy [[range 2-7]; prior ASCT, 76%; PI refractory only, 6%; IMiD refractory only, 12%; double

refractory to PI and IMiD, 65%) a 41% ORR was observed consisting of 3 very good partial responses and 4 PRs. Median time to response was 4 (range 4-8) weeks.

Conclusions:

SC DARA-PH20 was well tolerated and achieved serum trough concentrations similar to or greater than IV DARA with a lower rate of IRRs compared to IV DARA over a significantly shorter infusion time. Preliminary data suggest that in this pt population SC DARA-PH20 may enable similar response rates to IV DARA monotherapy. The 1800 mg dose level of DARA-PH20 was selected as the RP2D for part 2 of the study. These early data support further study of SC DARA in clinical trials.

Example 3. Development of co-formulations of daratumumab and hyaluronidase

Various co-formulations were evaluated in order to establish the overall physico-chemical stability and delivery of daratumumab and rHuPH20 in the co-formulated product. The impact of the concentrations of the active constituent and/or the excipients in the formulations was evaluated in some of the stability and/or animal studies (shelf stability, shaking stability and in pig infusion studies). **Table 8** provides a summary of the formulations that have been used in various studies.

Table 8.

Formulation	Daratumumab (mg/mL)	rHuPH20 (U/mL)	His (mM)	Sorbitol/ Sucrose (mM)	PS20 (%w/v)	Met (mg/mL)	pH
1	100	500	10	300	0.04	2	5.5
2	120	2000	10	300	0.04	1	5.6
3	100	500	10	300	0.0	2	5.5
4	100	500	10	300	0.01	2	5.5
5	100	500	10	300	0.02	2	5.5
6	100	500	10	300	0.06	2	5.5
7	100	0	10	200/ 100	0.04	0	5.5
8	100	0	10	100/ 200	0.04	0	5.5
9	100	50	10	300	0.04	1	5.5
10	100	500	10	300	0.04	1	5.5

11	100	2000	10	300	0.04	1	5.5
12	100	5000	10	300	0.04	1	5.5
His: histidine Met: methionine							

The ranges of the excipients and the active constituents in the tested formulations are shown in **Table 9.**

Table 9.

Formulation component	Range
rHuPH20	0-2000 U/mL
Daratumumab	100-120 mg/mL
Histidine	10 mM
Sorbitol	100-300 mM
Sucrose	0-200 mM
Polysorbate-20 (PS20)	0.0-0.04 % (w/v)
Methionine	0 - 2 mg/mL

The generated formulations were tested in various assays for their characteristics, including evaluation of sub-visible particles, micro flow imaging (MFI), size exclusion chromatography (SEC), capillary iso-electric focusing (cIEF), SDS-PAGE (non-reducing and reducing), peptide mapping, extractable volume, turbidity, osmolality, and pH.

Sub-visible particles (Sub-vis): Number of sub-visible particles sizes of $\geq$10 µm or $\geq$ 25 µm is usually aggregates of protein molecules and can be assayed by the light obscuration HIAC method whereby the solution is passed through a small orifice and the blockage of light provides the information on the particle size passing through.

MFI: An orthogonal to the light obscuration method, micro flow imaging (MFI) takes snapshot images of particles flowing through and re-converts back to the number of particles present in a particular volume of liquid. This method provides information about the large aggregates of proteins present in the solution.

SEC: A size exclusion chromatographic separation method whereby a column is used to distribute the molecules within the solution flowing through according to their broad size range. Monomers, aggregates and fragments elute at different times from the column and hence their relative proportions in a sample can be quantified using a standard UV detector.

cIEF: Capillary iso-electric focusing distributes the molecules according to the charge on the molecule and is a good indicator of the overall chemical stability. For example deamidation may result in a change in the charge of the molecule and thus would be picked up by this method. The method provides an idea of the total acidic, basic and intact % of molecules present in the solution.

SDS (reducing and non-reducing conditions): SDS method provides information on the physical stability of the molecule. SDS provides a measure of the intact, aggregated and fragmented species present in the solution. Non reducing SDS provides information on the respective intact, aggregated and fragmented constituents of the antibody while reducing SDS (after disulfide disruption) provides the same information for the heavy and light chains of the antibody.

Peptide mapping: Peptide mapping is an essential technique for studying the primary structure of proteins. For recombinant protein pharmaceuticals, peptide mapping is used for the initial proof of structure characterization. Peptide mapping also provides information on post translational modifications such as deamidation, oxidation etc

Extractable volume: The method provides information on the amount/volume of liquid that can be withdrawn from the vial after the respective time point.

Turbidity: A light scattering based method to evaluate the physical stability of the solution. An increase in the size of the particles or aggregates results in an increase in the light scattering signal and is hence picked up as turbidity (opalescence) of the solution. Turbidity is measured in Nephelometric Turbidity Units (NTU).

Osmolality: Provides a measure of the total osmotic activity which is dependent on the total true activity of the molecules (activity coefficient multiplied by concentration). The solution must be close to the osmolality of the serum to be injectable.

pH: Provides an idea of the overall stability and is important that the pH remains constant throughout the shelf life.

rHuPH20 enzymatic activity: The determination of hyaluronidase activity is based on the formation of a precipitate when hyaluronic acid (HA) binds with acidified serum. The activity is measured by incubating hyaluronidase with HA for 30 minutes in a 96-well plate format at 37°C and then precipitating the undigested HA with the addition of

acidified serum. The resulting turbidity is measured at 640 nm and the decrease in turbidity resulting from enzymatic cleavage of the HA substrate is a measure of the hyaluronidase activity.

Shelf stability of Formulation 1 (100 mg/mL Daratumumab, 10 mM Histidine, 300 mM Sorbitol, 0.04 % PS20, 2mg/mL Methionine, 500U/mL PrHuh20, pH 5.5) was evaluated using assays described. Samples were put on stability in 25R vials (filled at 16 mL volume) at different temperatures (5, 25 and 40°C) and vials were pulled for analysis using various assays at different time points (0, 1, 2, 3, 4, 5 and/or 6 months). Data indicates that the co-formulated product is stable under the storage conditions both with respect to the Daratumumab as well as rHuPH20 as indicated by various assays. The profile as observed for particles, color, turbidity, sec etc was very similar to well behaved stable antibodies and the data is comparable to the stability data of some commercial mAb formulations.

Table 10 shows the number of particles in Formulation 1 over time as assessed using HIAC.

Table 11 shows the number of particles in Formulation 1 over time as assessed using MFI.

Table 12 shows the pH of Formulation 1 over time.

Table 13 shows the turbidity of Formulation 1 over time.

Table 14 shows the proportion of high-molecular weight aggregates and low molecular weight fragments in Formulation 1 over time.

Table 15 shows the acidic and basic species in Formulation 1 over time as assessed using cIEF.

Table 16 shows the percent (%) purity of Formulation 1 over time as assessed using reduced SDS-PAGE.

Table 17 shows the percent (%) purity of Formulation 1 over time as assessed using non-reduced SDS-PAGE.

Table 18 shows the percent (%) bioactivity of daratumumab and enzyme activity of rhPH20 in Formulation 1 over time.

Table 10.

Storage Temperature (°C)	Average Cumulative Count / mL of particle sizes ≥10μM or ≥25μM

	0 months		3 months		6 months	
	≥ 10µm	≥ 25µm	≥ 10µm	≥ 25µm	≥ 10µm	≥ 25µm
5	7.17	6.33	6.5	4.8	42.50	30.83
25	7.17	6.33	5.5	1.8	110.50	81.83
40	7.17	6.33	64.5	44.8		

Table 11.

	0 months	3 months			6 months	
Temperature (°C)	5	5	25	40	5	25
Particles/mL ≥ 2 - < 10µm ECD	539	1155	3668	3371	686	3581
Particles/mL ≥ 10 - < 25µm ECD	9.3	39	93	80	36	105
Particles/mL ≥ 25 - < 70µm ECD	1.4	2.6	13	9.3	7.4	7.2
Particles/mL ≥ 70µm ECD	0.5	0.2	0.2	0.2	0.0	0.5

Table 12.

Storage Temperature (°C)	pH over Storage Time (months)				
	0m	1m	2m	3m	6m
5	5.7	NA	5.7	5.7	5.6
25	5.7	5.7	5.7	5.7	5.6
40	5.7	5.7	5.6	5.7	NA
m: month					
NA: not analyzed					

Table 13.

Storage Temperature (°C)	Average NTU over Storage Time (months)				
	0m	1m	2m	3m	6m
5	3.5		3.5	3.5	3.4
25	3.5	3.5	3.6	3.7	4.6
40	3.5	4	5.5	8.2	NA
m: month					
NTU: Nephelometric Turbidity Units					
NA: not analyzed					

Table 14.

		Percentage (%) of species		
Storage Time (months)	Storage Temperature (°C)	HMW	Monomer	LMW
0	5	0.74	99.25	0.01
	25	0.74	99.25	0.01
	40	0.74	99.25	0.01
3	5	0.84	99.16	0.02
	25	1.11	98.75	0.14
	40	1.87	94.50	3.64
6	5	0.89	99.10	NA
	25	1.30	98.38	NA
	40	NA	NA	NA
HMW: high molecular weight species				
LMW: low molecular weight species				
NA: not analyzed				

Table 15.

Storage	Storage	% Main peak	% Acidic	% Basic

Time (months)	Temperature (°C)		peaks	peaks
0	5	69.2	27.8	3
	25	69.2	27.8	3
	40	69.2	27.8	3
3	5	68.2	28.9	2.9
	25	63	32.8	4.2
	40	31.3	60.2	8.5
6	5	68.1	28.7	3.2
	25	55.4	38	6.6
	40	NA	NA	NA
NA: not analyzed				

Table 16

Storage Temperature (°C)	%Purity over Storage Time (in months)				
	0m	1m	2m	3m	6m
5	98.16	NA	97.96	98	98.02
25	98.16	97.87	97.66	97.57	96.75
40	98.16	96.4	95	92.88	NA
m: month					
NA: not analyzed					

Table 17.

Storage Temperature (°C)	%Purity over Storage Time (in months)				
	0	1	2	3	6
5	97.56		97.64	97.64	97.5
25	97.56	97.27	96.97	96.62	95.63
40	97.56	94.84	92.61	89.37	NA

m: month
NA: not analyzed

Table 18.

Molecule	Assay	Storage Time and Temperature		
		0 months	6 months	6 months
			5°C	25°C
Daratumumab	ADCC*	102	103	83
	CDC*	95	101	93
PH20	Enzymatic activity**	574	600 or 584?	609
*Percent control **U/mL				

Agitation (shaking) stability of the Formulation 1 was also assessed using the above assays to characterize the formulations and study the impact of PS concentrations by varying just PS20 concentrations in that formulation (Formulations 1, 3, 4, 5 and 6 in **Table 8** where PS20 was varied to 0, 0.01, 0.02, 0.04, 0.06 %). The data indicated that the co-formulation was stable under the shaking conditions both with respect to the Daratumumab as well as the enzyme as indicated by various assays. The profile as observed for particles, color, turbidity, sec etc was very similar to well behaved stable antibodies for all concentrations of PS but 0 % (0% PS20 formulation had particles and was not stable) and the data was comparable to the stability data of some commercial mAb formulations (data not shown).

Shelf stability of Formulation 2 (120 mg/mL daratumumab, 10 mM Histidine, 300 mM Sorbitol, 0.04 % PS20, 1 mg/mL Methionine, 2000U/mL rhuPH20, pH 5.5) was evaluated using assays described. Samples were put on stability in 25R vials filled at 13.27 mL volume with overfill (1500 mg dose) at different temperatures and vials were pulled for analysis using various assays as below. The collected data indicated that the co-formulated product is stable under the storage conditions both with respect to the daratumumab as wellrHuPH20. The profile as observed for particles, color, turbidity, sec

etc was very similar to well behaved stable antibodies and the data was comparable to the stability data of some commercial mAb formulations. rhuPH20 is very susceptible at higher temperatures and loses all activity very fast when stored at 40°C. **Table 19** shows the characteristics of the formulation.

Table 19.

	Storage Time, Temperature and relative humidity (RH)		
Characteristics and/or assay	0 months	1 month	1 month
	5°C	40°C/ 75%RH	25°C/ 60%RH
Average Cumulative Count / mL of particle sizes 2-10 µm	58.89	375.68	4285.81
Average Cumulative Count / mL of particle sizes 10-25 µm	3.34	12.96	1.98
Average Cumulative Count / mL of particle sizes ≥25 µm	1.23	.49	.12
PS20 (% w/v)	0.038	0.02	0.03
pH	5.6	5.6	5.6
Turbidity (NTU)	5	11	6
% Purity (cSDS, reducing)	98.4	98.6	98.1
% AGHC, aglycosylated heavy chain (cSDS, reducing)	0.4	0.5	0.4
% Purity (cSDS, non-reducing)	97.7	95.2	97.7
% monomer, SE-HPLC	99.1	98.0	98.8
% aggregate, SE-HPLC	0.9	1.6	1.1

% fragments SE-HPLC	<0.10	0.4	<0.10
Daratumumab bioactivity, CDC (% control)	105	88	99
Daratumumab bioactivity, ADCC (% control)	99	73	103
rhuPH20 activity (U/mL)	2205	0	2258

Formulations 3-8 were tested for their shelf stability or shaking stability using some or all assays described. The data indicated that the Formulations 3-8 were stable under the conditions assessed both with respect to the daratumumab as well as HuPH20 (formulations 7 and 8 had no rHuPH20). Methionine was included into formulations 1-6 and 9-12 to provide added oxidation stability. The profile as observed for particles, color, turbidity, sec etc was very similar to well behaved stable antibodies and the data was comparable to the stability data of some commercial mAb formulations (data not shown).

Agitation (shaking) stability of the Formulation 1 was also assessed using the above assays to characterize the formulations and study the impact of PS20 concentrations by varying just PS20 concentrations in that formulation (Formulations 1, 3, 4, 5 and 6 in **table 8** where PS20 was varied to 0, 0.01, 0.02, 0.04, 0.06 %). The data indicated that the co-formulation was stable under the shaking conditions both with respect to daratumumab as well as rHuPH20 as indicated by various assays. The profile as observed for particles, color, turbidity, sec etc was very similar to well behaved stable antibodies for all concentrations of PS but 0 % and the data was comparable to the stability data of some commercial mAb formulations (data not shown).

Formulations 9-12 were also evaluated for the assessment of subcutaneous administration of daratumumab with varying concentrations of enzyme in a swine model as described in Example 2. These studies were conducted to determine a suitable concentration of rhPH20 to deliver 16 mL of daratumumab. End points were infusion pressure, area of swelling or bleb if measurable and qualitative assessment of site. Dose dependent increase in infusion pressure was seen. All rhPH20 concentrations tested (50, 500, 2000, 5000 U/mL were sufficient to deliver 16 mL of daratumumab.

What is claimed is:

1. A pharmaceutical composition comprising daratumumab, histidine, sorbitol, and a surfactant, wherein the turbidity of the pharmaceutical composition is about 6 Nephelometric Turbidity Units (NTU) or less following storage for 1 month at 25°C and 60% relative humidity.

2. The pharmaceutical composition of claim 1, wherein the daratumumab in the pharmaceutical composition has a percentage (%) purity of at least 97.0% as measured by SDS-PAGE under non reducing conditions following storage for 1 month at 25°C and 60% relative humidity.

3. The pharmaceutical composition of claim 1 or 2, wherein at least 98% of the daratumumab in the pharmaceutical composition is present as a monomer following storage for 3 months at about 25°C and 60% relative humidity as measured by size exclusion separation.

4. The pharmaceutical composition of any one of claims 1-3, wherein about 1.11% or less of the daratumumab in the pharmaceutical composition is present as high-molecular weight aggregates as measured by size exclusion chromatographic separation following storage for 1 month at 25°C and 60% relative humidity.

5. The pharmaceutical composition of any one of claims 1-4, wherein the pharmaceutical composition comprises daratumumab at a concentration of about 20 mg/mL to about 120 mg/mL.

6. The pharmaceutical composition of any one of claims 1-5, wherein the pharmaceutical composition comprises daratumumab at a concentration of about 20 mg/mL.

7. The pharmaceutical composition of any one of claims 1-5, wherein the pharmaceutical composition comprises daratumumab at a concentration of about 120 mg/mL.

8. The pharmaceutical composition of any one of claims 1-7, wherein the pharmaceutical composition comprises histidine at a concentration of about 5 to about 50 mM.

9. The pharmaceutical composition of any one of claims 1-8, wherein the pharmaceutical composition comprises histidine at a concentration of about 10 mM.

10. The pharmaceutical composition of any one of claims 1-8, wherein the pharmaceutical composition comprises histidine at a concentration of about 8 mM.

11. The pharmaceutical composition of any one of claims 1-10, wherein the pharmaceutical composition comprises sorbitol at a concentration of about 100 mM to about 300 mM.

12. The pharmaceutical composition of any one of claims 1-11, wherein the pharmaceutical composition comprises sorbitol at a concentration of about 300 mM.

13. The pharmaceutical composition of any one of claims 1-11, wherein the pharmaceutical composition comprises sorbitol at a concentration of about 270 mM.

14. The pharmaceutical composition of any one of claims 1-13, wherein the pharmaceutical composition is at pH of 5.0 to 6.0.

15. The pharmaceutical composition of any one of claims 1-14, wherein the pharmaceutical composition is at pH of about 5.3-5.8.

16. The pharmaceutical composition of any one of claims 1-15, wherein the surfactant is polysorbate 20.

17. The pharmaceutical composition of claim 16, wherein the pharmaceutical composition comprises polysorbate 20 at a concentration of about 0.01% w/v to about 0.1% w/v.

18. The pharmaceutical composition of claim 16 or 17, wherein the pharmaceutical composition comprises polysorbate 20 at a concentration of about 0.04% w/v.

19. The pharmaceutical composition of any one of claims 1-18, further comprising an antioxidant.

20. The pharmaceutical composition of claim 19, wherein the antioxidant is methionine.

21. The pharmaceutical composition of claim 20, wherein the pharmaceutical composition comprises methionine at a concentration of about 0.1 mg/mL to about 2.5 mg/mL.

22. The pharmaceutical composition of claim 20 or 21, wherein the pharmaceutical composition comprises methionine at a concentration of about 1 mg/mL.