

FORMULATIONS OF CD38 ANTIBODIES AND USES THEREOF

FIELD OF THE INVENTION

Pharmaceutical compositions comprising anti-CD38 antibodies and their use as medicaments, *e.g.*, in treating or preventing cancer.

5 BACKGROUND OF THE INVENTION

CD38 is a type II transmembrane glycoprotein which is normally found on hematopoietic cells and at low levels in solid tissues. Expression of CD38 in hematopoietic cells depends on the differentiation and activation status of the cell. Lineage-committed hematopoietic cells express the protein, while it is lost by mature cells and expressed again on activated
10 lymphocytes. CD38 is also expressed on B cells, whereby plasma cells express particularly high levels of CD38. Approximately 80% of resting NK cells and monocytes express CD38 at lower levels, as do various other hematological cell types, including lymph node germinal center lymphoblasts, intrafollicular cells, dendritic cells, erythrocytes, and platelets (Lee and Aarhus 1993; Zocchi, Franco et al. 1993; Malavasi, Funaro et al. 1994; Ramaschi, Torti et al.
15 1996). With regards to solid tissues, CD38 is expressed in the gut by intraepithelial cells and lamina propria lymphocytes, by Purkinje cells and neurofibrillary tangles in the brain, by epithelial cells in the prostate, β -cells in the pancreas, osteoclasts in the bone, retinal cells in the eye, and sarcolemma of smooth and striated muscle.

CD38 is expressed in a large number of hematological malignancies. Expression has been
20 observed particularly in the malignant cells of multiple myeloma (MM) (Lin, Owens et al. 2004) and chronic lymphocytic leukemia (CLL) (Damle 1999), and was also reported in Waldenström's macroglobulinemia (Konoplev, Medeiros et al. 2005), primary systemic amyloidosis (Perfetti, Bellotti et al. 1994), mantle-cell lymphoma (Parry-Jones, Matutes et al. 2007), acute lymphoblastic leukemia (Keyhani, Huh et al. 2000), acute myeloid leukemia
25 (Marinov, Koubek et al. 1993; Keyhani, Huh et al. 2000), NK-cell leukemia (Suzuki, Suzumiya et al. 2004), NK/T-cell lymphoma (Wang, Wang et al. 2015) and plasma cell leukemia (van de Donk, Lokhorst et al. 2012).

Other diseases, where CD38 expression could be involved, include, *e.g.* broncho-epithelial carcinomas of the lung, breast cancer (evolving from malignant proliferation of epithelial
30 lining in ducts and lobules of the breast), pancreatic tumors, evolving from the β -cells (insulinomas), tumors evolving from epithelium in the gut (*e.g.* adenocarcinoma and squamous cell carcinoma), carcinoma in the prostate gland, seminomas in testis, ovarian cancers, and neuroblastomas. Other disclosures also suggest a role of CD38 in autoimmunity

such as Graves' disease and thyroiditis (Antonelli, Fallahi et al. 2001), type 1 and 2 Diabetes (Mallone and Perin 2006) and inflammation of airway smooth muscle cells during asthma (Deshpande, White et al. 2005). Moreover, CD38 expression has been associated with HIV infection (Kestens, Vanham et al. 1992; Ho, Hultin et al. 1993).

- 5 CD38 is a multifunctional protein. Functions ascribed to CD38 include both receptor mediation in adhesion and signaling events and (ecto-) enzymatic activity. As an ectoenzyme, CD38 uses NAD⁺ as substrate for the formation of cyclic ADP-ribose (cADPR) and ADPR, but also of nicotinamide and nicotinic acid-adenine dinucleotide phosphate (NAADP). cADPR has been shown to act as second messenger for Ca²⁺ mobilization from the endoplasmatic reticulum.
- 10 Several anti-CD38 antibodies are described in the literature, for instance in WO 2006/099875 A1, WO2008037257 A2, WO 2011/154453 A1, WO 2007/042309 A1, WO 2008/047242 A1, WO2012/092612 A1, Cotner, Hemler et al. 1981; Ausiello, Urbani et al. 2000; Lande, Urbani et al. 2002; de Weers, Tai et al. 2011; Deckert, Wetzel et al. 2014; Raab, Goldschmidt et al. 2015; Eissler, Filosto et al. 2018; Roepcke, Plock et al. 2018; and Schooten 2018.
- 15 CD38 antibodies may affect CD38 expressing tumor cells by one or more of the following mechanisms of action: complement-dependent cytotoxicity (CDC), antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), programmed cell death, trogocytosis, elimination of immune suppressor cells and modulation of enzymatic activity (van de Donk, Janmaat et al. 2016; Krejcik, Casneuf et al. 2016; Krejcik, Frerichs et al. 2017; Chatterjee, Daenthanasamak et al. 2018; van de Donk 2018). However, in 2014,
- 20 it was proposed that, no CD38 antibodies had been described that could induce effective CDC, ADCC, ADCP as well as effectively inhibit CD38 enzyme activity (Lammerts van Bueren, Jakobs et al. 2014).

- Optimization of the effector functions may improve the effectivity of therapeutic antibodies
- 25 for treating cancer or other diseases, e.g., to improve the ability of an antibody to elicit an immune response to antigen-expressing cells. Such efforts are described in, e.g., WO 2013/004842 A2; WO 2014/108198 A1; WO 2018/031258 A1; Dall'Acqua, Cook et al. 2006; Moore, Chen et al. 2010; Desjarlais and Lazar 2011; Kaneko and Niwa 2011; Song, Myojo et al. 2014; Brezski and Georgiou 2016; Sondermann and Szymkowski 2016; Zhang, Armstrong
- 30 et al. 2017; Wang, Mathieu et al. 2018.

Despite these and other efforts in the art, however, there is a need for CD38 therapeutic antibodies with modulated potencies, and formulations comprising such.

SUMMARY OF THE INVENTION

The present invention concerns compositions, such as pharmaceutical compositions, comprising CD38 antibody C, comprising an Fc region comprising a mutation in one or more amino acid residues selected from the group corresponding to E430, E345 or S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index.

So, in one aspect, the invention relates to a pharmaceutical composition comprising

- a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising
 - an antigen-binding region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7, and
 - an Fc region comprising a mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index;
 - b) 5-40 mM histidine or acetate;
 - c) 100 – 400 mM sorbitol or sucrose; and
 - d) a surfactant.
- 20 In one aspect, the present invention relates to a pharmaceutical composition comprising
- a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising
 - a heavy chain comprising a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, the amino acid residues being numbered according to the EU index, and
 - a light chain comprising a VL region comprising a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7;
 - b) 5-40 mM histidine or acetate;
 - c) 100 – 400 mM sorbitol or sucrose; and
 - d) a surfactant.

These and other aspect and embodiments of the invention are described in more detail below.

LEGENDS TO THE FIGURES

Figure 1 shows an amino acid sequence alignment using Clustal 2.1 software for human IgG1m(a), IgG1m(f), IgG2, IgG3 and IgG4 Fc segments corresponding to residues P247 to K447 in the human IgG1 heavy chains, wherein the amino acid residues are numbered according to the EU index as set forth in Kabat. The amino acid sequences shown correspond to residues 130 to 330 in the heavy chain constant regions of the allotypic variants of human IgG1 designated IgG1m(za) (SEQ ID NO:64; UniProt accession No. P01857), IgG1m(f) (SEQ ID NO:65), IgG1m(z) (SEQ ID NO:66), IgG1m(a) (SEQ ID NO:67) and IgG1m(x) (SEQ ID NO:68); residues 126 to 326 of the IgG2 heavy chain constant region (SEQ ID NO:79; UniProt accession No. P01859); residues 177 to 377 of the IgG3 heavy chain constant region (SEQ ID NO:80; UniProt accession No. P01860), and residues 127 to 327 of the IgG4 heavy chain constant region (SEQ ID NO:81; UniProt accession No. P01861).

Figure 2 shows the binding of CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G to CD38 expressing NALM16 cells in comparison to CD38 antibodies IgG1-A, IgG1-B, IgG1-C and isotype control antibody. For more details, see Example 2.

Figure 3 shows the binding of CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G to CD38 expressed on cynomolgus PBMCs (A) or Daudi cells expressing high copy numbers of human CD38 (B) in comparison to isotype control antibody. For more details, see Example 2.

Figure 4 shows the percentage lysis induced by CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G of Ramos (A), Daudi (B), Wien-133 (C), NALM-16 (D), REH (E), RS4;11 (F), U266 (G) and RC-K8 (H) tumor cell lines in a CDC assay as compared to CD38 antibodies IgG1-A, IgG1-B and IgG1-C. For more details, see Example 3.

Figure 5 shows the effect of CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G on the number of viable NK cells (A), T cells (B) and B cells (C) in a CDC assay performed on whole blood as compared to CD38 antibodies IgG1-A, IgG1-B and IgG1-C. For more details, see Example 3.

Figure 6 shows the percentage lysis of Daudi cells induced by CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G in a chromium-release ADCC assay as compared to CD38 antibodies IgG1-A, IgG1-B, IgG1-C and isotype control antibody. For more details, see Example 4.

Figure 7 shows the dose-dependent FcγRIIIa cross-linking of CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G in an ADCC reporter assay as compared to CD38 antibodies IgG1-A, IgG1-B, IgG1-C and isotype control antibody. For more details, see Example 4.

- 5 Figure 8 shows the effect of CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G on the percentage of PKH-29^{pos}, CD14^{pos} and CD19^{neg} macrophages in an ADCP assay as compared CD38 antibodies IgG1-A, IgG1-B, IgG1-C and isotype control antibody. For more details, see Example 5.

- 10 Figure 9 shows the percentage lysis induced by CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G of Ramos (A), Daudi (B, C), Wien-133 (D, E) and NALM-16 (F, G) tumor cells lines in an apoptosis assay conducted with (C, E, G) or without (A, B, D, F) Fc-cross-linking antibody, as compared to CD38 antibodies IgG1-A, IgG1-B, IgG1-C and isotype control antibody. For more details, see Example 6.

Figure 10 illustrates the enzymatic activities of CD38.

- 15 Figure 11 shows the effect of CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G on the cyclase activity of HisCD38 (A), Daudi cells (B) and Wien-133 cells (C) as reflected by % NDG conversion over time, in comparison to CD38 antibodies IgG1-A, IgG1-B, IgG1-C and isotype control antibody.

- 20 Figure 12 shows the effect of CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G on the CD38 expression on Daudi cells after 45 minute co-culture with macrophages in comparison to CD38 antibodies IgG1-A, IgG1-B, IgG1-C and isotype control antibody. Macrophages were from Donor A (A, B) or Donor B (B, D) and antibody opsonized cells were tested for CD38 expression (A, B) or human IgG staining (C, D).

- 25 Figure 13 shows the effect of CD38 antibodies IgG1-B-E430G and IgG1-C-E430G on the CD38 expression on T regulatory cells with or without PBMCs, in comparison to IgG1-B.

Figure 14 shows the percentage lysis induced by CD38 antibodies IgG1-A-E430G (closed triangles), IgG1-B-E430G (closed circles) and IgG1-C-E430G (closed squares) of different B cell tumor cell lines in a CDC assay as compared to CD38 antibodies IgG1-B (open circle) and isotype control antibody (open diamonds). For more details, see Example 3.

- 30 Figure 15 shows a summary of some of the EC50 values depicted in Table 4. EC50 values of CDC induced by antibodies IgG1-B, IgG1-B-E430G and IgG1-C-E430G on 20 different B cell

tumor cell lines are shown. Each square, triangle or circle represents a different B cell tumor cell line. EC50 values obtained with AML cell lines were not included because IgG1-B-E430G was not tested on AML cell lines.

5 Figure 16 shows the percentage lysis induced by CD38 antibody IgG1-C-E430G (closed circles) of different AML tumor cell lines in a CDC assay as compared to CD38 antibodies IgG1-B (open circles) and isotype control antibody (closed squares). For more details, see Example 3.

10 Figure 17 shows the percentage lysis induced by CD38 antibodies IgG1-B-E430G (closed circles) and IgG1-C-E430G (closed squares) of T regulatory cells in a CDC assay as compared to CD38 antibodies IgG1-B (open circles). For more details, see Example 3.

Figure 18 shows the percentage lysis of Daudi, Wien-133, Granta 519 and MEC-2 cells induced by CD38 antibodies IgG1-B-E430G, IgG1-C-E430G in a chromium-release ADCC assay as compared to CD38 antibodies IgG-B, IgG1-C and IgG1-b12-E430G. For more details, see Example 4.

15 Figure 19 shows the dose-dependent FcγRIIIa cross-linking of CD38 antibodies IgG1-A-E430G, IgG1-B-E430G and IgG1-C-E430G in an ADCC reporter assay with T regulatory cells as compared to CD38 antibodies IgG1-A, IgG1-B, IgG1-C and isotype control antibody. For more details, see Example 4.

20 Figure 20 shows the tumor size (mm³) in mice treated with either CD38 antibody IgG1-C-E430G or PBS (negative control). For more details see Example 9.

25 Figure 21 illustrates the assay setup to measure trogocytosis. 1) Daudi cells were labelled with PKH-26 (membrane staining) and cell trace violet (cytosol staining) and opsonized with CD38 antibodies. 2) Labelled Daudi cells and macrophages were co-incubated for 2h at 37 °C to allow macrophage attachment. 3) Cell membrane transfer or trogocytosis from Daudi cells to macrophages. 4) Detachment of the macrophage-Daudi interaction and degradation of the Daudi cell membrane in the macrophage. For more details see Example 8.

Figure 22 shows complement-mediated cytotoxicity by IgG1-C-E430G or Darzalex® in bone marrow mononuclear cells from 3 newly diagnosed MM patients (A, B and D) and 1 relapsed/refractory MM patient (C).

30 Figure 23 shows the percentage lysis induced by CD38 antibody IgG1-C-E430G (closed circles) of different MM tumor cell lines in a CDC assay as compared to CD38 antibodies IgG1-

B (open circles) and isotype control antibody (closed squares). For more details, see Example 3.

DETAILED DESCRIPTION OF THE INVENTION

5 In describing the embodiments of the invention specific terminology will be resorted to for the sake of clarity. However, the invention is not intended to be limited to the specific terms so selected, and it is understood that each specific term includes all technical equivalents which operate in a similar manner to accomplish a similar purpose.

10 The present invention relates to pharmaceutical compositions comprising an antibody comprising at least the VH CDR1-3 and VL CDR1-3 of anti-CD38 antibody C, and an Fc region comprising a mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain.

As shown in Example 3, CDC was enhanced for all three tested CD38 IgG1 antibodies – A, B and C - upon introduction of an E430G mutation. Surprisingly, however, the magnitude of CDC enhancement differed between the antibody clones tested. Without the E430G mutation, 15 IgG1-B was already a good inducer of CDC, whereas IgG1-C and IgG1-A induced modest and no CDC respectively. Nonetheless, after introduction of the E430G mutation, however, IgG1-C-E430G induced more effective CDC compared to IgG1-B-E430G. In tumor cells and T regulatory cells that had lower CD38 expression levels, EC50 values of IgG1-C-E430G were lower than those of IgG1-B-E430G.

20 The antibody may also demonstrate ADCC. For example, as shown in Example 4, IgG1-C achieved a higher maximum percent lysis as compared to IgG1-B in the ⁵¹Cr release assay and an increased FcγRIIIa binding in the ADCC reporter assay as compared to IgG1-B. Introduction of the E430G mutation reduced the maximum percent lysis in the ⁵¹Cr release assay and the FcγRIIIa binding in the ADCC reporter assay for all three antibodies. IgG1-C- 25 E430G induced a similar maximum percent lysis as compared to IgG1-B-E430G and IgG1-A-E430G in the ⁵¹Cr release assay and similar FcγRIIIa binding in the ADCC reporter assay.

Moreover, the ability of an anti-CD38 antibody to inhibit CD38 cyclase activity can be retained. For example, as shown in Example 7, IgG1-C-E430G displayed stronger inhibition of CD38 cyclase activity compared to IgG1-B-E430G, the former resulting in an inhibition of 30 about 40% and the latter about 25%. Without being limited to theory, a stronger inhibition of CD38 cyclase activity may reduce production of cADPR, a potent second messenger that regulate Ca²⁺ mobilization from the cytosol, which in turn may lead to decreased Ca²⁺ mobilization and reduced signaling of downstream pathways that control various biological

processes, such as proliferation and insulin secretion. Without being limited to theory, a stronger inhibition of CD38 cyclase activity may thus affect, e.g., reduce, the ability of immune suppressor cells to suppress an immune response.

Other functionalities that can be modulated include trogocytosis. Specifically, CD38 expression on Daudi cells was significantly reduced by co-culture with macrophages and CD38 antibody; however, the reduction in CD38 expression was strongest with E430G mutated antibody (Example 8). Surprisingly, CD38 expression on T regulatory cells co-cultured with PBMCs was only reduced after incubation with E430G-mutated CD38 antibody; no reduction in CD38 expression was found when T regulatory cells were incubated with antibody B. Without being limited to theory, the ability of antibodies to induce trogocytosis of CD38-expressing, non-cancerous immune cells, particularly immunosuppressive cells, may in a cancer patient result in an increased immune response against tumor cells, irrespective of whether the tumor cells express CD38 or not.

The antibody may also be able to kill tumor cells in vivo as shown in Example 9, where two weekly doses of IgG1-C-E430G reduced the tumor growth in two out of five tested DLBCL PDX models that had highest CD38 mRNA expression.

Definitions

As used herein, the term "CD38" generally refers to human CD38 (UniProtKB - P28907 (CD38_HUMAN)) having the sequence set forth in SEQ ID NO:38, but may also, unless contradicted by context, refer to variants, isoforms and orthologs thereof. Variants of human CD38 with S274, Q272R, T237A or D202G mutations are described in WO 2006/099875 A1 and WO 2011/154453 A1.

The term "immunoglobulin" refers to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) low molecular weight chains and one pair of heavy (H) chains, all four potentially inter-connected by disulfide bonds. The structure of immunoglobulins has been well characterized. See for instance Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Briefly, each heavy chain typically is comprised of a heavy chain variable (VH) region and a heavy chain constant (CH) region. The CH region typically is comprised of three domains, CH1, CH2, and CH3. The heavy chains are typically inter-connected via disulfide bonds in the so-called "hinge region". Each light chain typically is comprised of a light chain variable (VL) region and a light chain constant region, the latter typically comprised of one domain, CL. The VH and VL regions may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed

complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each VH and VL region is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see also Chothia and Lesk J. Mol. Biol. 196, 901 917 (1987)).

Unless otherwise stated or contradicted by context, CDR sequences herein are identified according to IMGT rules using DomainGapAlign (Lefranc MP., Nucleic Acids Research 1999;27:209-212 and Ehrenmann F., Kaas Q. and Lefranc M.-P. Nucleic Acids Res., 38, D301-307 (2010); see also internet http address www.imgt.org/).

Unless otherwise stated or contradicted by context, reference to amino acid positions in the CH or Fc region/Fc domain in the present invention is according to the EU-numbering (Edelman et al., Proc Natl Acad Sci U S A. 1969 May;63(1):78-85; Kabat et al., Sequences of proteins of immunological interest. 5th Edition - 1991 NIH Publication No. 91-3242). An amino acid residue in a CH of another isotype than human IgG1 may, however, alternatively be referred to by the corresponding amino acid position in a wild-type human IgG1 heavy chain in which the amino acid residues are numbered according to the EU index. Specifically, the corresponding amino acid position can be identified as illustrated in Figure 1, i.e., by (a) aligning the amino acid sequence of the non-IgG1 constant region (or a segment thereof) with the amino acid sequence of a human IgG1 heavy chain (or segment thereof) in which the amino acid residues are numbered according to the EU index, and (b) identifying which amino acid position in the IgG1 heavy chain the amino acid residue is aligned with. Accordingly, the position of such an amino acid residue can herein be referred to as "the amino acid residue at a position corresponding to", followed by the amino acid position in a wild-type human IgG1 heavy chain numbered according to the EU index. When referring to one or more of a number of different amino acid positions, this can be referred to herein as "a mutation in one or more amino acid residues at positions selected from the group consisting of the positions corresponding to", "a mutation in one or more amino acid residues at positions corresponding to" or simply "a mutation in one or more amino acid residues selected from the group corresponding to", followed by two or more amino acid positions (e.g., E430, E345 and S440) in a human wild-type IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index.

The term "hinge region" as used herein is intended to refer to the hinge region of an immunoglobulin heavy chain. Thus, for example the hinge region of a human IgG1 antibody corresponds to amino acids 216-230 according to the EU numbering.

The term "CH2 region" or "CH2 domain" as used herein is intended to refer to the CH2 region of an immunoglobulin heavy chain. Thus, for example the CH2 region of a human IgG1 antibody corresponds to amino acids 231-340 according to the EU numbering. However, the CH2 region may also be any of the other subtypes as described herein.

- 5 The term "CH3 region" or "CH3 domain" as used herein is intended to refer to the CH3 region of an immunoglobulin heavy chain. Thus, for example the CH3 region of a human IgG1 antibody corresponds to amino acids 341-447 according to the EU numbering. However, the CH3 region may also be any of the other subtypes as described herein.

10 The term "antibody" (Ab) in the context of the present invention refers to an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative of either thereof, which has the ability to specifically bind to an antigen. The antibody of the present invention comprises an Fc region (also referred to herein as "Fc domain") of an immunoglobulin and an antigen-binding region. An antibody generally contains two CH2-CH3 regions and a connecting region, e.g. a hinge region, e.g. at least an Fc domain. Thus, the antibody of the present invention may comprise an Fc region and an antigen-binding region. The variable regions of the heavy and light chains of the immunoglobulin molecule contain a binding domain that interacts with an antigen. The constant or "Fc" regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as effector cells) and components of the complement system such as C1q, the first component in the classical pathway of complement activation. As used herein, unless contradicted by context, the Fc region of an immunoglobulin typically contains at least a CH2 domain and a CH3 domain of an immunoglobulin CH, and may comprise a connecting region, e.g., a hinge region. An Fc-region is typically in dimerized form via, e.g., disulfide bridges connecting the two hinge regions and/or non-covalent interactions between the two CH3 regions. The dimer may be a homodimer (where the two Fc region monomer amino acid sequences are identical) or a heterodimer (where the two Fc region monomer amino acid sequences differ in one or more amino acids). Preferably, the dimer is a homodimer. An Fc region-fragment of a full-length antibody can, for example, be generated by digestion of the full-length antibody with papain, as is well-known in the art. An antibody as defined herein may, in addition to an Fc region and an antigen-binding region, further comprise one or both of an immunoglobulin CH1 region and a CL region. An antibody may also be a multispecific antibody, such as a bispecific antibody or similar molecule. The term "bispecific antibody" refers to an antibody having specificities for at least two different, typically non-overlapping, epitopes. Such epitopes may be on the same or different targets. If the epitopes are on different targets, such targets may be on the same cell or different cells or cell types. As indicated above, unless otherwise stated or clearly contradicted by the context, the term antibody herein includes fragments of an antibody which comprise at least

a portion of an Fc-region and which retain the ability to specifically bind to the antigen. Such fragments may be provided by any known technique, such as enzymatic cleavage, peptide synthesis and recombinant expression techniques. It has been shown that the antigen-binding function of an antibody may be performed by fragments of a full-length antibody.

5 Examples of binding fragments encompassed within the term "Ab" or "antibody" include, without limitation, monovalent antibodies (described in WO2007059782 by Genmab); heavy-chain antibodies, consisting only of two heavy chains and naturally occurring in e.g. camelids (e.g., Hamers-Casterman (1993) *Nature* 363:446); ThioMabs (Roche, WO2011069104), strand-exchange engineered domain (SEED or Seed-body) which are asymmetric and

10 bispecific antibody-like molecules (Merck, WO2007110205); Triomab (Pharma/Fresenius Biotech, Lindhofer et al. 1995 *J Immunol* 155:219; WO2002020039); Fc Δ Adp (Regeneron, WO2010151792), Azymetric Scaffold (Zymeworks/Merck, WO2012/058768), mAb-Fv (Xencor, WO2011/028952), Xmab (Xencor), Dual variable domain immunoglobulin (Abbott, DVD-Ig, U.S. Patent No. 7,612,181); Dual domain double head antibodies (Unilever; Sanofi

15 Aventis, WO20100226923), Di-diabody (ImClone/Eli Lilly), Knobs-into-holes antibody formats (Genentech, WO9850431); DuoBody (Genmab, WO 2011/131746); Bispecific IgG1 and IgG2 (Pfizer/ Rinat, WO11143545), DuetMab (MedImmune, US2014/0348839), Electrostatic steering antibody formats (Amgen, EP1870459 and WO 2009089004; Chugai, US201000155133; Oncomed, WO2010129304A2); bispecific IgG1 and IgG2 (Rinat

20 neurosciences Corporation, WO11143545), CrossMAbs (Roche, WO2011117329), LUZ-Y (Genentech), Biclonic (Merus, WO2013157953), Dual Targeting domain antibodies (GSK/Domantis), Two-in-one Antibodies or Dual action Fabs recognizing two targets (Genentech, NovImmune, Adimab), Cross-linked Mabs (Karmanos Cancer Center), covalently fused mAbs (AIMM), CovX-body (CovX/Pfizer), FynomAbs (Covagen/Janssen ilag), DutaMab

25 (Dutalys/Roche), iMab (MedImmune), IgG-like Bispecific (ImClone/Eli Lilly, Shen, J., et al. *J Immunol Methods*, 2007. 318(1-2): p. 65-74), TIG-body, DIG-body and PIG-body (Pharmabcine), Dual-affinity retargeting molecules (Fc-DART or Ig-DART, by MacroGenics, WO/2008/157379, WO/2010/080538), BEAT (Glenmark), Zybodies (Zyngenia), approaches with common light chain (Crucell/ Merus, US7262028) or common heavy chains (K λ Bodies by

30 NovImmune, WO2012023053), as well as fusion proteins comprising a polypeptide sequence fused to an antibody fragment containing an Fc-region like scFv-fusions, like BsAb by ZymoGenetics/BMS, HERCULES by Biogen Idec (US007951918), SCORPIONS by Emergent BioSolutions/Trubion and Zymogenetics/BMS, Ts2Ab (MedImmune/AZ (Dimasi, N., et al. *J Mol Biol*, 2009. 393(3): p. 672-92), scFv fusion by Genentech/Roche, scFv fusion by Novartis, scFv fusion by Immunomedics, scFv fusion by Changzhou Adam Biotech Inc (CN 102250246), TvAb by Roche (WO 2012025525, WO 2012025530), mAb² by f-Star (WO2008/003116), and dual scFv-fusion s. It should be understood that the term antibody, unless otherwise specified, includes monoclonal antibodies (such as human monoclonal antibodies), polyclonal antibodies, chimeric antibodies, humanized antibodies, monospecific antibodies (such as

bivalent monospecific antibodies), bispecific antibodies, antibodies of any isotype and/or allotype; antibody mixtures (recombinant polyclonals) for instance generated by technologies exploited by Symphogen and Merus (Oligoclomics), multimeric Fc proteins as described in WO2015/158867, and fusion proteins as described in WO2014/031646. While these different antibody fragments and formats are generally included within the meaning of antibody, they collectively and each independently are unique features of the present invention, exhibiting different biological properties and utility.

A "CD38 antibody" or "anti-CD38 antibody" as described herein is an antibody which binds specifically to the antigen CD38.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations, insertions or deletions introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

The terms "monoclonal antibody", "monoclonal Ab", "monoclonal antibody composition", "mAb", or the like, as used herein refer to a preparation of Ab molecules of single molecular composition. The Ab molecules of a monoclonal antibody composition may be monospecific, displaying a single binding specificity and affinity for a particular epitope, or multi-specific, such as bispecific. Accordingly, the term "human monoclonal antibody" refers to monospecific or multi-specific Abs which have variable and constant regions derived from human germline immunoglobulin sequences. Human mAbs may be generated by a hybridoma which includes a B cell obtained from a transgenic or trans-chromosomal non-human animal, such as a transgenic mouse or rat, having a genome comprising a human heavy chain transgene repertoire and a light chain transgene repertoire, rearranged to produce a functional human antibody and fused to an immortalized cell. Human mAbs may also be generated by recombinant means.

As used herein, "isotype" refers to the immunoglobulin class that is encoded by heavy chain constant region genes, including, for instance, IgG1, IgG2, IgG3, IgG4, IgD, IgA1, IgA2, IgE, and IgM, as well as any allotypes thereof such as IgG1m(z), IgG1m(a), IgG1m(x), IgG1m(f) and mixed allotypes thereof such as IgG1m(za), IgG1m(zax), IgG1m(fa), etc. (see, for instance, de Lange, Experimental and Clinical Immunogenetics 1989;6(1):7-17).

Further, each heavy chain isotype can be combined with either a kappa (κ) or lambda (λ) light chain. The term "mixed isotype" used herein refers to Fc region of an immunoglobulin generated by combining structural features of one isotype with the analogous region from another isotype thereby generating a hybrid isotype. A mixed isotype may comprise an Fc region having a sequence comprised of two or more isotypes selected from the following IgG1, IgG2, IgG3, IgG4, IgD, IgA1, IgGA2, IgE, or IgM thereby generating combinations such as e.g. IgG1/IgG3, IgG1/IgG4, IgG2/IgG3, IgG2/IgG4 or IgG1/IgA.

The term "full-length antibody" when used herein, refers to an antibody which contains all heavy and light chain constant and variable domains corresponding to those that are normally found in a wild-type antibody of the isotype in question, except for any indicated mutations.

A "full-length bivalent, monospecific monoclonal antibody" when used herein, refers to a bivalent, monospecific antibody formed by a pair of identical HCs and a pair of identical LCs, with the constant and variable domains corresponding to those normally found in an antibody of the particular isotype in question, except for any indicated mutations.

The term "antigen-binding region", "antigen binding region", "binding region" or antigen binding domain, as used herein, refers to a region of an antibody which is capable of binding to the antigen. This binding region is typically defined by the VH and VL domains of the antibody which may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). The antigen can be any molecule, such as a polypeptide, e.g. present on a cell.

The term "target", as used herein, refers to a molecule to which the antigen binding region of the antibody binds. The target includes any antigen towards which the raised antibody is directed. The term "antigen" and "target" may in relation to an antibody be used interchangeably and constitute the same meaning and purpose with respect to any aspect or embodiment of the present invention.

The term "epitope" means a protein determinant capable of specific binding to an antibody variable domain. Epitopes usually consist of surface groupings of molecules such as amino acids, sugar side chains or a combination thereof and usually have specific three-dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. The epitope may comprise amino acid residues

directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding.

5 A "variant" as used herein refers to a protein or polypeptide sequence which differs in one or more amino acid residues from a reference sequence. A variant may, for example, have a sequence identity of at least 80%, such as 90%, or 95%, or 97%, or 98%, or 99%, to a reference sequence. Also or alternatively, a variant may differ from the reference sequence by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutation(s) such as substitutions, insertions or deletions of amino acid residues.

10 In the context of the present invention, conservative substitutions may be defined as substitutions within the following classes of amino acids:

- Acidic Residues: Asp (D) and Glu (E)
- Basic Residues: Lys (K), Arg (R), and His (H)
- Hydrophilic Uncharged Residues: Ser (S), Thr (T), Asn (N), and Gln (Q)
- Aliphatic Uncharged Residues: Gly (G), Ala (A), Val (V), Leu (L), and Ile (I)
- 15 - Non-polar Uncharged Residues: Cys (C), Met (M), and Pro (P)
- Aromatic Residues: Phe (F), Tyr (Y), and Trp (W)

Alternative conservative amino acid residue substitution classes:

1. A S T
2. D E
- 20 3. N Q
4. R K
5. I L M
6. F Y W

Alternative Physical and Functional Classifications of Amino Acid Residues:

- Alcohol group-containing residues: S and T
- Aliphatic residues: I, L, V, and M
- Cyclo-alkenyl-associated residues: F, H, W, and Y
- 5 - Hydrophobic residues: A, C, F, G, H, I, L, M, R, T, V, W, and Y
- Negatively charged residues: D and E
- Polar residues: C, D, E, H, K, N, Q, R, S, and T
- Positively charged residues: H, K, and R
- Small residues: A, C, D, G, N, P, S, T, and V
- 10 - Very small residues: A, G, and S
- Residues involved in turn formation: A, C, D, E, G, H, K, N, Q, R, S, P, and T
- Flexible residues: Q, T, K, S, G, N, D, E, and R

"Sequence identity" as used herein refers to the percent identity between two sequences as a function of the number of identical positions shared by the sequences (i.e., percent homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The percent identity between two nucleotide or amino acid sequences may e.g. be determined using the algorithm of E. Meyers and W. Miller, Comput. Appl. Biosci 4, 11-17 (1988) that has been incorporated into the ALIGN program (version 2.0), using a PAM 120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences may be determined using the Needleman and Wunsch, J. Mol. Biol. 48, 444-453 (1970) algorithm. Other tools for sequence alignments are publicly available on the internet, and include, without limitation, Clustal Omega and EMBOSS Needle on the EMBL-EBI website www.ebi.ac.uk. Typically, default settings can be used.

In the context of the present invention the following notations are, unless otherwise indicated, used to describe a mutation; name of amino acid which is mutated, followed by the position number which is mutated, followed by what the mutation encompasses. Thus, if the mutation is a substitution, the name of the amino acid which replaces the prior amino acid is included, if the amino acid is deleted it is indicated by a "*", if the mutation is an addition the amino acid being added is included after the original amino acid. Amino acid names may be one or three-letter codes. Thus, for example; the substitution of a glutamic acid in position 430 with a glycine is referred to as E430G, substitution of glutamic acid in position 430 with any amino acid is referred to as E430X, deletion of glutamic acid in position 430 is referred to as E430* and addition of a proline after glutamic acid at position E430 is referred to as E430EP.

As used herein, "immunosuppressive cells" refer to immune cells which may suppress an immune response in a subject, such as by suppressing the activity of effector T cells and/or inhibiting T cell proliferation. Examples of such immunosuppressive cells include, but are not limited to, regulatory T cells (Tregs), regulatory B cells (Bregs) and myeloid-derived suppressor cells (MDSCs). There are also immunosuppressive NK cells, NKT cells, macrophages and antigen-presenting cells (APCs). An example of a phenotype for an immunosuppressive NK cell is CD56^{bright}CD16⁻.

"Regulatory T cells" or "Tregs" or "Treg" refers to T lymphocytes that regulate the activity of other T cell(s) and/or other immune cells, usually by suppressing their activity. An example of a Treg phenotype is CD3⁺CD4⁺CD25⁺CD127^{dim}. Tregs may further express Foxp3. It is appreciated that Tregs may not be fully restricted to this phenotype.

"Effector T cells" or "Teffs" or "Teff" refers to T lymphocytes that carry out a function of an immune response, such as killing tumor cells and/or activating an antitumor immune-response which can result in clearance of the tumor cells from the body. Examples of Teff phenotypes include CD3⁺CD4⁺ and CD3⁺CD8⁺. Teffs may secrete, contain or express markers such as IFN γ , granzyme B and ICOS. It is appreciated that Teffs may not be fully restricted to these phenotypes.

"Myeloid-derived suppressor cells" or "MDSCs" or "MDSC" refers to a specific population of cells of the hematopoietic lineage that express the macrophage/monocyte marker CD11b and the granulocyte marker Gr-1/Ly-6G. An example of an MDSC phenotype is CD11b⁺HLA-DR⁻CD14⁻CD33⁺CD15⁺. MDSCs typically also show low or undetectable expression of the mature antigen presenting cell markers MHC Class II and F480. MDSCs are immature cells of the myeloid lineage and may further differentiate into other cell types, such as macrophages, neutrophils, dendritic cells, monocytes or granulocytes. MDSCs may be found naturally in

normal adult bone marrow of human and animals or in sites of normal hematopoiesis, such as the spleen.

"Regulatory B cell" or "Breg" or "Bregs" refers to B lymphocytes that suppress immune responses. An example of a Breg phenotype is CD19⁺CD24⁺CD38⁺. Bregs may suppress immune responses by inhibiting T cell proliferation mediated by IL-10 secreted by the Bregs. It is appreciated that other Breg subsets exist, and are described in for example Ding et al., (2015) Human Immunology 76: 615-621.

As used herein, the term "effector cell" refers to an immune cell which is involved in the effector phase of an immune response. Exemplary immune cells include a cell of a myeloid or lymphoid origin, for instance lymphocytes (such as B cells and T cells including cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes, eosinophils, polymorphonuclear cells, such as neutrophils, granulocytes, mast cells, and basophils. Some effector cells express Fc receptors (FcRs) or complement receptors and carry out specific immune functions. In some embodiments, an effector cell such as, e.g., a natural killer cell, is capable of inducing ADCC. For example, monocytes, macrophages, neutrophils, dendritic cells and Kupffer cells which express FcRs, are involved in specific killing of target cells and/or presenting antigens to other components of the immune system, and/or binding to cells that present antigens. In some embodiments the ADCC can be further enhanced by antibody driven classical complement activation resulting in the deposition of activated C3 fragments on the target cell. C3 cleavage products are ligands for complement receptors (CRs), such as CR3, expressed on myeloid cells. The recognition of complement fragments by CRs on effector cells may promote enhanced Fc receptor-mediated ADCC. In some embodiments, antibody driven classical complement activation leads to C3 fragments on the target cell. These C3 cleavage products may promote direct complement-dependent cellular cytotoxicity (CDCC). In some embodiments, an effector cell may phagocytose a target antigen, target particle or target cell which may depend on antibody binding and mediated by FcγRs expressed by the effector cells. The expression of a particular FcR or complement receptor on an effector cell may be regulated by humoral factors such as cytokines. For example, expression of FcγRI has been found to be up-regulated by interferon γ (IFN γ) and/or G-CSF. This enhanced expression increases the cytotoxic activity of FcγRI-bearing cells against targets. An effector cell can phagocytose a target antigen or phagocytose or lyse a target cell. In some embodiments, antibody driven classical complement activation leads to C3 fragments on the target cell. These C3 cleavage products may promote direct phagocytosis by effector cells or indirectly by enhancing antibody mediated phagocytosis.

The term "Fc effector functions," as used herein, is intended to refer to functions that are a consequence of binding a polypeptide or antibody to its target, such as an antigen, on a cell

membrane wherein the Fc effector function is attributable to the Fc region of the polypeptide or antibody. Examples of Fc effector functions include (i) C1q-binding, (ii) complement activation, (iii) complement-dependent cytotoxicity (CDC), (iv) antibody-dependent cell-mediated cytotoxicity (ADCC), (v) Fc-gamma receptor-binding, (vi) antibody-dependent cellular phagocytosis (ADCP), (vii) complement-dependent cellular cytotoxicity (CDCC), (viii) complement-enhanced cytotoxicity, (ix) binding to complement receptor of an opsonized antibody mediated by the antibody, (x) opsonisation, (xi) trogocytosis, and (xii) a combination of any of (i) to (xi).

As used herein, the term "complement activation" refers to the activation of the classical complement pathway, which is initiated by a large macromolecular complex called C1 binding to antibody-antigen complexes on a surface. C1 is a complex, which consists of 6 recognition proteins C1q and a hetero-tetramer of serine proteases, C1r2C1s2. C1 is the first protein complex in the early events of the classical complement cascade that involves a series of cleavage reactions that starts with the cleavage of C4 into C4a and C4b and C2 into C2a and C2b. C4b is deposited and forms together with C2a an enzymatic active convertase called C3 convertase, which cleaves complement component C3 into C3b and C3a, which forms a C5 convertase. This C5 convertase splits C5 into C5a and C5b and the last component is deposited on the membrane and that in turn triggers the late events of complement activation in which terminal complement components C5b, C6, C7, C8 and C9 assemble into the membrane attack complex (MAC). The complement cascade results in the creation of pores in the cell membrane which causes lysis of the cell, also known as complement-dependent cytotoxicity (CDC). Complement activation can be evaluated by using C1q efficacy, CDC kinetics CDC assays (as described in WO2013/004842, WO2014/108198) or by the method *Cellular deposition of C3b and C4b* described in Beurskens et al., J Immunol April 1, 2012 vol. 188 no. 7, 3532-3541.

The term "complement-dependent cytotoxicity" (CDC), as used herein, is intended to refer to the process of antibody-mediated complement activation leading to lysis of the cell to which the antibody is bound, which, without being bound by theory is believed to be the result of pores in the membrane that are created by the assembly of the so-called membrane attack complex (MAC). Suitable assays for evaluating CDC are known in the art and include, for example, *in vitro* assays in which normal human serum is used as a complement source, as described in Example 3. A non-limiting example of an assay for determining the maximum lysis of CD38 expressing cells as mediated by a CD38 antibody, or the EC50 value, may comprise the steps of:

(a) plating about 100,000 CD38-expressing cells in 40 μ L culture medium supplemented with 0.2% BSA per well in a multi-well plate;

- (b) preincubating cells for 20 minutes with 40 μ L of serially diluted CD38 antibody (0.0002-10 μ g/mL);
- (c) incubating each well for 45 minutes at 37°C with 20 percent of pooled normal human serum;
- 5 (d) adding a viability dye and measuring the percentage of cell lysis on a flow cytometer;
- (e) determining the maximum lysis and/or calculating the EC50 value using non-linear regression.

The term "antibody-dependent cell-mediated cytotoxicity" ("ADCC") as used herein, is intended to refer to a mechanism of killing of antibody-coated target cells by cells expressing Fc
10 receptors that recognize the constant region of the bound antibody. Suitable assays for evaluating ADCC are known in the art and include, for example, the assays described in Example 4. Non-limiting examples of assays for determining the ADCC of CD38-expressing cells as mediated by a CD38 antibody may comprise the steps of the ^{51}Cr -release assay or the reporter assay set out below.

15 ADCC with ^{51}Cr release assay

- (a) plating about 5,000 ^{51}Cr labelled CD38-expressing cells (e.g., Daudi cells) in 50 μ L culture medium supplemented with 0.2% BSA per well in a multi-well plate;
- (b) preincubating cells for 15 minutes with 50 μ L of serially diluted CD38 antibody (0.0002-10 μ g/mL);
- 20 (c) incubating each well for 4 hours at 37°C with 500,000 freshly isolated peripheral blood mononuclear cells (PBMCs) per well;
- (d) measuring the amount of ^{51}Cr release in 75 μ L supernatant on a gamma counter;
- (e) calculating the percentage of cell lysis as (cpm sample - cpm spontaneous lysis)/(cpm maximal lysis - cpm spontaneous lysis) wherein cpm is counts per minute.

25 ADCC with reporter assay

- (a) plating about 5,000 CD38-expressing cells (e.g., Daudi cells) in 10 μ L in multi-well plates suitable for optical readings (e.g., 384-well OptiPlates from PerkinElmer Inc.) in a standard medium (e.g., RPMI 1640) supplemented with 25% low IgG serum;
- 5 (b) incubating each well for 6 hours at 37°C with 10 μ L engineered Jurkat cells stably expressing the Fc γ RIIIa receptor, V158 (high affinity) variant, and an NFAT response element driving expression of firefly luciferase as effector cells and 10 μ L serially diluted CD38 antibody (0.0002-10 μ g/mL);
- (c) incubating each well 5 minutes at RT with 30 μ L Luciferase substrate and measuring luminescence.
- 10 The term "antibody-dependent cellular phagocytosis" ("ADCP") as used herein is intended to refer to a mechanism of elimination of antibody-coated target cells by internalization by phagocytes. The internalized antibody-coated target cells are contained in a vesicle called a phagosome, which then fuses with one or more lysosomes to form a phagolysosome. Suitable assays for evaluating ADCP are known in the art and include, for example, the in vitro
- 15 cytotoxicity assay with macrophages as effector cells and video microscopy as described by van Bij et al. in *Journal of Hepatology* Volume 53, Issue 4, October 2010, Pages 677–685, and the in vitro cytotoxicity assay described in Example 5. A non-limiting example of an assay for determining the ADCP of CD38 expressing cells as mediated by a CD38 antibody may comprise the steps of:
- 20 (a) differentiating freshly isolated monocytes to macrophages with 5 days incubation in GM-CSF-containing medium;
- (b) plating about 100,000 macrophages per well in a multi-well plate in dendritic cell medium with GM-CSF;
- 25 (c) adding 20,000 CD38-antibody opsonized CD38-expressing cells (e.g., Daudi cells), labelled with a generic fluorescent membrane dye, per well for 45 minutes at 37°C;
- (d) measuring the percentage of CD14-positive, CD19-negative, membrane-dye-positive macrophages on a flow cytometer.

As used herein, "trocytosis" refers to a process characterized by the transfer of cell surface molecules from a donor cell to an acceptor cell, such as an effector cell. Typical acceptor cells

30 include T and B cells, monocytes/macrophages, dendritic cells, neutrophils, and NK cells. Trocytosis-mediated transfer of a cell surface molecule such as, e.g., CD38, from a donor

cell to an acceptor cell may also result in the transfer of an antibody-antigen complex from the donor cell to an acceptor cell, *i.e.*, an antibody-antigen complex where an antibody is bound to the cell surface molecule. In particular, a specialized form of trogocytosis may occur when the acceptor cells are Fc-gamma-receptor (FcγR) expressing effector cells; these

5 acceptor cells may take up and internalize donor cell-associated immune complexes composed of specific antibodies bound to target antigens on donor cells, typically after binding of FcγRs to the Fc regions of the antibodies. Suitable assays for evaluating trogocytosis are known in the art and include, for example, the assay in Example 8. Non-

10 limiting examples of assays for determining trogocytosis of CD38 expressing cells as mediated by a CD38 antibody include the following:

Trogocytosis (Daudi cells):

- (a') differentiating freshly isolated monocytes to macrophage with 5 days GM-CSF;
- (b') plating about 100,000 macrophages per well in dendritic cell medium with GM-CSF;
- (c') adding about 20,000 CD38 antibody-opsonized Daudi cells, labelled with a generic
- 15 fluorescent membrane dye, per well for 45 minutes at 37°C;
- (d') measuring CD38 expression on Daudi cells on a flow cytometer, wherein a reduction in CD38 on CD38-antibody opsonized Daudi cells as compared to a control indicates trogocytosis.

Trogocytosis (Tregs):

- (a) plating about 500,000 freshly isolated PBMCs per well in cell culture medium O/N at
- 20 37°C;
- (b) adding about 100,000, CD38 antibody-opsonized Tregs, labelled with a generic fluorescent intracellular amine dye, per well overnight (O/N) at 37°C; and
- (c) measuring CD38 expression on Tregs on a flow cytometer, wherein a reduction in
- 25 CD38 on CD38-antibody opsonized Tregs as compared to a control indicates trogocytosis.

The control can be selected by the skilled person based on the specific purpose of the study or assay in question. However, non-limiting examples of controls include (i) the absence of any antibody and (ii) an isotype control antibody. One example of an isotype control antibody

is antibody b12, having the VH and VL sequences described in Table 1. In some embodiments where it is desired to evaluate the trogocytosis-effect of an antibody as described herein, the control may be (iii) a reference antibody having a different antigen-binding region and/or a different Fc region.

- 5 In some embodiments, in step (b), in addition or alternative to the fluorescent intracellular amine dye, the Tregs are labelled with a generic fluorescent membrane dye.

In some embodiments, in step (d') and (c) of the trogocytosis assays outlined above, the reduction in CD38 antibody on the donor cells can also be measured. For example, in cases where the CD38 antibody is a human IgG (huIgG) antibody, a secondary antibody can be
10 used to detect huIgG.

In addition to Daudi cells (ATCC CCL-213), tumor cells suitable for the first assay include, without limitation, those listed in Table 2, particularly those with a high CD38 expression.

In addition to Tregs, suitable CD38-expressing cells for the second assay include immune cells such as, e.g., NK cells, B cells, T cells and monocytes, as well as tumor cells listed in
15 Table 2, particularly those with a low CD38 expression level.

The term "vector," as used herein, is intended to refer to a nucleic acid molecule capable of inducing transcription of a nucleic acid segment ligated into the vector. One type of vector is a "plasmid", which is in the form of a circular double stranded DNA loop. Another type of vector is a viral vector, wherein the nucleic acid segment may be ligated into the viral
20 genome. Certain vectors are capable of autonomous replication in a host cell into which they are introduced (for instance bacterial vectors having a bacterial origin of replication and episomal mammalian vectors). Other vectors (such as non-episomal mammalian vectors) may be integrated into the genome of a host cell upon introduction into the host cell, and thereby are replicated along with the host genome. Moreover, certain vectors are capable of
25 directing the expression of genes to which they are operatively linked. Such vectors are referred to herein as "recombinant expression vectors" (or simply, "expression vectors"). In general, expression vectors of utility in recombinant DNA techniques are often in the form of plasmids. In the present specification, "plasmid" and "vector" may be used interchangeably as the plasmid is the most commonly used form of vector. However, the present invention is
30 intended to include such other forms of expression vectors, such as viral vectors (such as replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions.

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which one or more expression vectors have been introduced. For example, the HC and LC of a CD38 antibody as described herein may both be encoded by the same expressing vector, and a host cell transfected with the expression vector. Alternatively, the HC and LC of a CD38 antibody as described herein may be encoded by different expression vectors, and a host cell co-transfected with the expression vectors. It should be understood that the term "host cell" is intended to refer not only to the particular subject cell, but also to the progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but are still included within the scope of the term "host cell" as used herein. Recombinant host cells include, for example, transfectomas, such as CHO cells, HEK-293 cells, PER.C6, NS0 cells, and lymphocytic cells, and prokaryotic cells such as *E. coli* and other eukaryotic hosts such as plant cells and fungi.

The term "transfectoma", as used herein, includes recombinant eukaryotic host cells expressing the Ab or a target antigen, such as CHO cells, PER.C6, NS0 cells, HEK-293 cells, plant cells, or fungi, including yeast cells.

The term "treatment" refers to the administration of an effective amount of a pharmaceutical composition of the present invention with the purpose of easing, ameliorating, arresting or eradicating (curing) symptoms or disease states.

The term "effective amount" or "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of an antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the pharmaceutical composition are outweighed by the therapeutically beneficial effects.

Specific embodiments of the invention

In one aspect, there is provided a pharmaceutical composition comprising

(a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising

- an antigen-binding region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7, and

- an Fc region comprising a mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index;
- (b) 5-40 mM histidine or acetate;
- 5 (c) 100 – 400 mM sorbitol or sucrose; and
- (d) a surfactant.

In one aspect, there is provided a pharmaceutical composition consisting essentially of

- (a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising
 - an antigen-binding region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7, and
 - an Fc region comprising a mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index;
- (b) 5-40 mM histidine or acetate;
- 15 (c) 100 – 400 mM sorbitol or sucrose; and
- (d) a surfactant.

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- (a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising
 - an antigen-binding region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7, and
 - an Fc region comprising a mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index;
- (b) 5-40 mM histidine or acetate;
- 25 (c) 100 – 400 mM sorbitol or sucrose; and
- (d) a surfactant,
- 30

in aqueous solution.

In one aspect, there is provided a pharmaceutical composition comprising

- 35 a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising

- a heavy chain comprising a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, the amino acid residues being numbered according to the EU index, and
- a light chain comprising a VL region comprising a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7;
- b) 5-40 mM histidine or acetate;
- c) 100 – 400 mM sorbitol or sucrose; and
- d) a surfactant.

In one aspect, there is provided a pharmaceutical composition consisting essentially of

- a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising
 - a heavy chain comprising a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, the amino acid residues being numbered according to the EU index, and
 - a light chain comprising a VL region comprising a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7;
- b) 5-40 mM histidine or acetate;
- c) 100 – 400 mM sorbitol or sucrose; and
- d) a surfactant.

In one aspect, there is provided a pharmaceutical composition consisting of

- a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising
 - a heavy chain comprising a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, the amino acid residues being numbered according to the EU index, and
 - a light chain comprising a VL region comprising a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7;
- b) 5-40 mM histidine or acetate;
- c) 100 – 400 mM sorbitol or sucrose; and
- d) a surfactant,

in aqueous solution.

In some embodiments of a pharmaceutical composition of the invention, a) may be from 1 to 80 mg/mL, such as 1 to 60 mg/ml, 1 to 40 mg/mL, 1 to 30 mg/ml or 1 to 25 mg/ml; 2 to 80 mg/mL, such as 2 to 40 mg/mL or 2 to 30 mg/ml; or 10 to 80 mg/mL, such as 10 to 40 mg/mL or 10 to 30 mg/ml; or 15 to 80 mg/ml, such as 15 to 40 mg/ml, such as 15 to 25 mg/ml, such as 2 mg/ml, 4 mg/mL, 6 mg/mL, 8 mg/mL, 10 mg/mL, 12 mg/mL, 14 mg/mL, 16 mg/mL, 18 mg/mL, 20 mg/mL, 22 mg/mL, 24 mg/mL, 26 mg/mL, 28 mg/mL, 30 mg/mL, 32 mg/mL, 34 mg/mL, 36 mg/mL, 38 mg/mL, 40 mg/mL, or 50 mg/mL of the antibody. In one embodiment, a) is about 20 mg/mL of the antibody. In a particularly contemplated embodiment, a) is 20 mg/mL of the antibody.

In some embodiments of a pharmaceutical composition of the invention, b) may be from 5 to 30 mM, such as 5 to 25 mM, such as 10 mM, 11, mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM or 30 mM of histidine or acetate. In one embodiment, b) is about 20 mM, such as 20 mM, of histidine or acetate. In one specific embodiment, b) is acetate. In a particularly contemplated embodiment, b) is histidine.

In some embodiments of a pharmaceutical composition of the invention, c) may be from 100 to 350 mM, such as 100 to 300 mM, 100 to 260 mM, 100 to 200 mM, 150 to 350 mM, 200 to 300 mM, 200 to 260 mM, 200 to 350 mM, 200 to 300 mM, 200 to 260 mM, 230 to 350 mM, 230 to 300 mM, 230 to 260 mM or 240 to 260 mM; such as 245 mM, 246 mM, 247 mM, 248 mM, 249 mM, 250 mM, 251 mM, 252 mM, 253 mM, 254 mM, or 255 mM of sorbitol or sucrose. In one embodiment, c) is about 250 mM, such as 250 mM, of sorbitol or sucrose. In one embodiment, c) is sucrose. In a particularly contemplated embodiment, c) is sorbitol.

The pharmaceutical composition may, for example, have a pH from 5.0 to 6.5, such as 5.5 to 6.5, such as 5.6 to 6.5, 5.7 to 6.5, 5.8 to 6.5, 5.9 to 6.5, 6.0 to 6.5, 5.5 to 6.4, 5.5 to 6.3, 5.5 to 6.2, 5.5 to 6.1, 5.5 to 6.0, 5.7 to 6.3, 5.8 to 6.2, 5.9 to 6.1. In one embodiment, the pH is about 6. In one embodiment, the pH is 6, such as 6.0.

Surfactants suitable for the pharmaceutical composition are known in the art and may, for example, be selected from the group comprising glycerol monooleate, benzethonium chloride, sodium docusate, phospholipids, polyethylene alkyl ethers, sodium lauryl sulfate and tricaprylin, benzalkonium chloride, citrimide, cetylpyridinium chloride and phospholipids, alpha tocopherol, glycerol monooleate, myristyl alcohol, phospholipids, poloxamers, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbintan fatty acid esters, polyoxyethylene sterarates, polyoxyl hydroxystearate, polyoxylglycerides, polysorbates,

propylene glycol dilaurate, propylene glycol monolaurate, sorbitan esters sucrose palmitate, sucrose stearate, tricaprylin and TPGS. In one particular embodiment, the surfactant is a polysorbate. Preferably, the surfactant is polysorbate 20 or 80. In one embodiment, the surfactant is polysorbate 20 (PS20). In a particularly contemplated embodiment, the surfactant is polysorbate 80 (PS80).

The concentration of the surfactant is typically from about 0.005% to 0.5% w/v, such as from about 0.01 to 0.1 % w/v, such as from about 0.01 to 0.09 % w/v such as from about 0.01 to 0.06 % w/v such as from about 0.01 to 0.05% w/v such as 0.02% w/v or 0.03% w/v or 0.04% w/v or 0.05% w/v, or 0.06% w/v. In one embodiment, the concentration of the surfactant is about 0.04% w/v, such as 0.04% w/v.

In a one embodiment, the pharmaceutical composition has a pH of 5.9 to 6.1, such as about 6 or 6.0, and comprises or consists essentially of:

- a) 1 to 80 mg/mL of the antibody
- b) 15 to 40 mM histidine
- c) 200 to 300 mM sorbitol
- d) 0.01% to 0.1 % w/v of a surfactant.

In one embodiment, the pharmaceutical composition has a pH of 5.9 to 6.1, such as about 6 or 6.0 and comprises or consists essentially of:

- a) 10 to 40 mg/mL of the antibody
- b) 15 to 40 mM histidine
- c) 200 to 300 mM sorbitol
- d) 0.02% to 0.06% w/v of a surfactant.

In one embodiment, the pharmaceutical composition has a pH of 5.9 to 6.1, such as about 6 or 6.0 and comprises or consists essentially of:

- a) 10 to 40 mg/mL of the antibody
- b) 15 to 25 mM histidine
- c) 240 to 260 mM sorbitol
- d) 0.02% to 0.06% w/v of a surfactant.

In a particular embodiment, the surfactant in d) is polysorbate, such as polysorbate 20 or polysorbate 80. In one embodiment, the surfactant is polysorbate 20. In one particularly contemplated embodiment, the surfactant is polysorbate 80.

A pharmaceutical composition according to the invention can be stable for a prolonged period of time, such as for a period of at least 8 weeks, such as for a period of 1-60 months, 1-48

months, 1-36 months, 1-30 months, 1-24 months, 1-18 months, 1-12 months or 1-6 months; such as for 2-60 months, 2-48 months, 2-36 months, 2-30 months, 2-24 months, 2-18 months, 2-12 months or 2-6 months; such as for 3-60 months, 3-48 months, 3-36 months, 3-30 months, 3-24 months, 3-18 months, 3-12 months or 3-6 months, such as for 48 months, 36 months, 30 months, 24 months, 18 months, 12 months, or 6 months. Preferably, the pharmaceutical composition is stable for such a prolonged period of time when the composition is kept at $5^{\circ}\text{C}\pm 3^{\circ}\text{C}$, $25^{\circ}\text{C}\pm 5^{\circ}\text{C}$ or $40^{\circ}\text{C}\pm 10^{\circ}\text{C}$, such as at $5^{\circ}\text{C}\pm 3^{\circ}\text{C}$, about $25^{\circ}\text{C}\pm 5^{\circ}\text{C}$ or about 40°C , such as at $5^{\circ}\text{C}\pm 3^{\circ}\text{C}$.

The stability of the pharmaceutical composition is also typically determined when the composition is kept at $5^{\circ}\text{C}\pm 3^{\circ}\text{C}$, $25^{\circ}\text{C}\pm 5^{\circ}\text{C}$ or $40^{\circ}\text{C}\pm 10^{\circ}\text{C}$, such as at $5^{\circ}\text{C}\pm 3^{\circ}\text{C}$, about $25^{\circ}\text{C}\pm 5^{\circ}\text{C}$ or about 40°C , such as at $5^{\circ}\text{C}\pm 3^{\circ}\text{C}$. However, the stability of the pharmaceutical composition can also or alternatively be determined when stored at $25^{\circ}\text{C}/60\%$ relative humidity (RH) and/or when stored at $40^{\circ}\text{C}/75\%\text{RH}$.

Methods for determining the stability of a pharmaceutical composition comprising an antibody are well known in the art, typically determining the change in one or more stability parameters from an initial time point to one or more later, predetermined time point(s). Preferably, however, stability is assessed by one or more of

- (a) sub-visible particle count;
- (b) pH;
- (c) protein concentration;
- (d) size-exclusion chromatography;
- (e) isoelectric focusing;
- (f) electrophoresis under reducing conditions;
- (g) electrophoresis under non-reducing conditions; and/or
- (h) visual inspection.

For example, stability may be assessed by determining

- (a) sub-visible particle count using a micro-flow imaging (MFI) microscope, optionally as provided in Example 12;
- (b) pH, optionally as provided in Example 11;
- (c) protein concentration using absorbance at 280 nm (A_{280}) optionally as provided in Example 11;

- (d) relative amount of antibody monomer as compared to the sum of antibody monomer, high molecular weight (HMW) species and low molecular weight (LMW) species present in the composition using size exclusion chromatography, optionally as provided in Example 11;
- 5 (e) the relative amount of antibody as compared to the sum of antibody, acidic variants of the antibody and basic variants of the antibody by imaged capillary isoelectric focusing (icIEF), optionally as provided in Example 11;
- (f) the relative amount of the sum of the light chain and heavy chain as compared to the sum of the light chain, heavy chain and non-glycosylated heavy chain by
10 reduced capillary electrophoresis, optionally as provided by Example 11;
- (g) the relative amount of antibody peak as compared to the sum of antibody, peaks with molecular weights lower than the antibody peak and peaks with molecular weights higher than the antibody peak by non-reduced capillary electrophoresis, optionally as provided by Example 11; and/or
- 15 (h) visual appearance, optionally as provided in Example 11.

One particular example is a pharmaceutical composition having a pH of about 6 and comprising or consisting essentially of

- a) about 20 mg/mL of the antibody,
b) about 20 mM histidine,
20 c) about 250 mM sorbitol, and
d) about 0.04% w/v of polysorbate 80.

Another particular example is a pharmaceutical composition having a pH of 6 and comprising, consisting or consisting essentially of

- a) 20 mg/mL of the antibody,
25 b) 20 mM histidine,
c) 250 mM sorbitol, and
d) 0.04% w/v of polysorbate 80,

in aqueous solution.

Another particular example is a pharmaceutical composition having a pH of about 6 and
30 comprising or consisting essentially of

- a) about 20 mg/mL of the antibody,
b) about 20 mM histidine,
c) about 250 mM sorbitol, and
d) about 0.04% w/v of polysorbate 20.

Another particular example is a pharmaceutical composition having a pH of 6 and comprising, consisting or consisting essentially of

- 5 a) 20 mg/mL of the antibody,
 b) 20 mM histidine,
 c) 250 mM sorbitol, and
 d) 0.04% w/v of polysorbate 20,

in aqueous solution.

Another particular example is a pharmaceutical composition having a pH of about 6 and comprising or consisting essentially of

- 10 a) about 20 mg/mL of the antibody,
 b) about 20 mM histidine,
 c) about 250 mM sucrose, and
 d) about 0.04% w/v of polysorbate 80.

Another particular example is a pharmaceutical composition having a pH of 6 and comprising, consisting or consisting essentially of

- 15 a) 20 mg/mL of the antibody,
 b) 20 mM histidine,
 c) 250 mM sucrose, and
 d) 0.04% w/v of polysorbate 80,

20 in aqueous solution.

Another particular example is a pharmaceutical composition having a pH of about 5.5 and comprising or consisting essentially of

- 25 a) about 20 mg/mL of the antibody,
 b) about 20 mM acetate,
 c) about 250 mM sorbitol, and
 d) about 0.04% w/v of polysorbate 80.

Another particular example is a pharmaceutical composition having a pH of about 5.5 and comprising, consisting or consisting essentially of

- 30 a) 20 mg/mL of the antibody,
 b) 20 mM acetate,
 c) 250 mM sorbitol, and
 d) 0.04% w/v of polysorbate 80,

in aqueous solution.

Another particular example is a pharmaceutical composition having a pH of about 6 and comprising or consisting essentially of

- a) about 20 mg/mL of the antibody,
- b) about 20 mM histidine,
- 5 c) about 250 mM sucrose, and
- d) about 0.04% w/v of polysorbate 20.

Another particular example is a pharmaceutical composition having a pH of 6 and comprising, consisting or consisting essentially of

- a) 20 mg/mL of the antibody,
- 10 b) 20 mM histidine,
- c) 250 mM sucrose, and
- d) 0.04% w/v of polysorbate 20,

in aqueous solution.

Another particular example is a pharmaceutical composition having a pH of about 5.5 and comprising or consisting essentially of

- a) about 20 mg/mL of the antibody,
- b) about 20 mM acetate,
- 15 c) about 250 mM sorbitol, and
- d) about 0.04% w/v of polysorbate 20.

Another particular example is a pharmaceutical composition having a pH of 5.5 and comprising, consisting or consisting essentially of

- a) 20 mg/mL of the antibody,
- b) 20 mM acetate,
- c) 250 mM sorbitol, and
- 25 d) 0.04% w/v of polysorbate 20,

in aqueous solution.

Another particular example is a pharmaceutical composition having a pH of about 5.5 and comprising or consisting essentially of

- a) about 20 mg/mL of the antibody,
- 30 b) about 20 mM acetate,
- c) about 250 mM sucrose, and
- d) about 0.04% w/v of polysorbate 80.

Another particular example is a pharmaceutical composition having a pH of 5.5 and comprising, consisting or consisting essentially of

- 5 a) 20 mg/mL of the antibody,
 b) 20 mM acetate,
 c) 250 mM sucrose, and
 d) 0.04% w/v of polysorbate 80,

in aqueous solution.

Another particular example is a pharmaceutical composition having a pH of about 5.5 and comprising or consisting essentially of

- 10 a) about 20 mg/mL of the antibody,
 b) about 20 mM acetate,
 c) about 250 mM sucrose, and
 d) about 0.04% w/v of polysorbate 20.

Another particular example is a pharmaceutical composition having a pH of 5.5 and comprising, consisting or consisting essentially of

- 15 a) 20 mg/mL of the antibody,
 b) 20 mM acetate,
 c) 250 mM sucrose, and
 d) 0.04% w/v of polysorbate 20,

20 in aqueous solution.

The invention also provides a pharmaceutical composition having a pH of about 6 and comprising or consisting essentially of

- 25 a) about 20 mg/mL of an antibody binding to human CD38,
 b) about 20 mM histidine,
 c) about 250 mM sorbitol, and
 d) about 0.04% w/v of polysorbate 80,

wherein the antibody is a full-length bivalent antibody comprising, consisting, or consisting essentially of two heavy chains and two light chains, wherein

- 30 - each heavy chain comprises a VH region and a CH region, wherein the VH region comprises SEQ ID NO:1 and the CH region comprises SEQ ID NO:24 or SEQ ID NO:46, and
 - each light chain comprises a VL region and a CL region, wherein the VL region comprises SEQ ID NO:5 and the CL region comprises SEQ ID NO:37.

In one embodiment, the pharmaceutical composition has a pH of about 6 and comprises, consists or consists essentially of

- a) 20 mg/mL of the antibody,
 - b) 20 mM histidine,
 - 5 c) 250 mM sorbitol, and
 - d) 0.04% w/v of polysorbate 80,
- in aqueous solution.

The pharmaceutical composition may be administered to a subject or patient by any suitable route and mode. In one embodiment, a pharmaceutical composition of the present invention is administered parenterally. "Administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and include epidermal, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, intratendinous, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection and infusion.

In one embodiment the pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion. In a particular embodiment, the pharmaceutical composition is administered by intravenous injection or infusion.

In some embodiments, the pharmaceutical composition according to the present invention is a concentrate to be diluted, typically prior to or in connection with administration to a subject or patient. Suitable diluents are known in the art. Preferred diluents include, without limitation, saline (0.9% NaCl) and dextrose (e.g., 5% w/v) in aqueous solution.

The invention also relates to kit-of-parts for simultaneous, separate or sequential use in therapy comprising a pharmaceutical composition according to the invention, optionally wherein the kit-of-parts contains more than one dosage of the pharmaceutical composition.

In one embodiment, the kit-of-parts comprises a pharmaceutical composition according to the invention in one or more containers, preferably vials.

In one embodiment, the kit-of-parts comprises more than one pharmaceutical composition according to the invention, for simultaneous, separate or sequential use in therapy.

The pharmaceutical composition according to any aspect or embodiment herein may comprise a CD38 antibody further characterized by other or additional features, as described below.

CD38 Antibodies

Antigen-binding region and variable regions

The antigen-binding region comprises one or more antibody variable domains allowing for specific binding to CD38, such as a VH region and a VL region. Similarly, the heavy and light chains comprise a VH and VL region, respectively. In the following, reference to sequences in the antigen-binding region may similarly apply to sequences of the heavy and/or light chain of an antibody according to the present invention.

Advantageously, the CDRs, VH region and/or VL region are similar or identical to those of antibody C, as set forth in Table 1. Preferably, the antigen-binding region, and/or the heavy and/or light chains comprises the CDRs of antibody C, set forth as SEQ ID NO:2 (VH-3003-C_CDR1), SEQ ID NO:3 (VH-3003-C_CDR2), SEQ ID NO:4 (VH-3003-C_CDR3), SEQ ID NO:6 (VL-3003-C_CDR1), AAS (VL-3003-C_CDR2) and SEQ ID NO:7 (VL-3003-C_CDR3).

So, in some embodiments, the antibody comprises an antigen-binding region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7.

In some embodiments, the antibody comprises a heavy chain comprising a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, and a light chain comprising a VL region comprising a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7.

In preferred embodiments, the antibody comprises the VH and VL region amino acid sequences of antibody C, i.e., the VH region comprises the sequence of SEQ ID NO:1 (VH-3003-C) and the VL region comprises the sequence of SEQ ID NO:5 (VL-3003-C).

However, it is well known in the art that mutations in the VH and VL of an antibody can be made to, for example, increase the affinity of an antibody to its target antigen, reduce its potential immunogenicity and/or to increase the yield of antibodies expressed by a host cell. Accordingly, in some embodiments, antibodies comprising mutations in the CDR, VH and/or VL sequences of antibody C are also contemplated. Particularly, such an antibody may differ in one or more amino acids as compared to the VH and/or VL sequence of antibody C, e.g., in

one or more CDRs, but its antigen-binding region preferably retains at least a substantial proportion (at least about 50 percent, 60 percent, 70 percent, 80 percent, 90 percent, 95 percent or more) of the affinity and/or specificity of antibody C. Typically, the VH and/or VL sequence of such antibodies retain significant sequence identity to the VH and/or VL sequence of antibody C. For example, the VH and/or VL region sequence of the antibody may differ from the corresponding VH and/or VL region sequence of antibody C by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutation(s) such as substitutions, insertions or deletions of amino acid residues. In some embodiments, the VH and/or VL and/or CDR region sequences of such antibodies may differ from the corresponding VH and/or VL and/or CDR regions sequences of antibody C mainly by conservative amino acid substitutions; for instance, 12, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 of the amino acid substitutions in the variant can be conservative. In some cases, an antibody comprising mutations in the VH and/or VL of antibody C may be associated with greater affinity and/or specificity than antibody C. For the purpose of the present invention, antibodies comprising mutations in the VH and/or VL region sequences of antibody C which, in comparison to antibody C, allow for a retained or improved affinity and specificity of the antibody in its binding to CD38 are particularly preferred.

For example, WO 2011/154453 A1 discloses CD38 antibodies comprising suitable mutations in CDR, VH and VL region amino acid sequences, where the amino acid residues at certain positions differ from those in the CDRs, VH and VL of antibody C as shown in Table 1. These positions thus represent candidate positions where mutations in the CDR, VH and VL sequences can be made while retaining or improving affinity and specificity of the antibody in its binding to CD38. In particular, positions in the VH and VL CDRs that can be mutated in the VH and VL of antibody C are indicated in SEQ ID NOS:40 to 43.

So, in some embodiments, one or more specific mutations are made in the CDRs as set forth in SEQ ID NOS:40 to 43, i.e., the VH and/or VL region comprises mutations in the CDRs as set forth in one or more of SEQ ID NO:40 (VH CDR1), SEQ ID NO:41 (VH CDR2), SEQ ID NO:42 (VH CDR3), and SEQ ID NO:44 (VL CDR3). The VH and VL regions of such an antibody may optionally maintain the original framework regions of antibody C. In one specific embodiment, the antigen-binding region comprises the CDRs as set forth in SEQ ID NO:40 wherein X_1 is S (VH CDR1), SEQ ID NO:41 wherein X_1 is R, X_2 is K, X_3 is A (VH CDR2), SEQ ID NO:42 wherein X_1 is A, X_2 is D and X_3 is V (VH CDR3), SEQ ID NO:43 (VL CDR1), AAS (VL CDR2) and SEQ ID NO:44 wherein X_1 is S (VL CDR3). In one specific embodiment, the antigen-binding region comprises the CDRs as set forth in SEQ ID NO:40 wherein X_1 is R (VH CDR1), SEQ ID NO:41 wherein X_1 is V, X_2 is K, X_3 is T (VH CDR2), SEQ ID NO:42 wherein X_1 is T, X_2 is A and X_3 is F (VH CDR3), SEQ ID NO:43 (VL CDR1), AAS (VL CDR2) and SEQ ID NO:44 wherein X_1 is N (VL CDR3). In one specific embodiment, the antigen-binding region

comprises the CDRs as set forth in SEQ ID NO:40 wherein X_1 is S (VH CDR1), SEQ ID NO:41 wherein X_1 is R, X_2 is K, X_3 is T (VH CDR2), SEQ ID NO:42 wherein X_1 is A, X_2 is D and X_3 is V (VH CDR3), SEQ ID NO:43 (VL CDR1), AAS (VL CDR2) and SEQ ID NO:44 wherein X_1 is S (VL CDR3). In one specific embodiment, the antigen-binding region comprises the CDRs as set forth in SEQ ID NO:40 wherein X_1 is R (VH CDR1), SEQ ID NO:41 wherein X_1 is V, X_2 is K, X_3 is V (VH CDR2), SEQ ID NO:42 wherein X_1 is T, X_2 is A and X_3 is F (VH CDR3), SEQ ID NO:43 (VL CDR1), AAS (VL CDR2) and SEQ ID NO:44 wherein X_1 is N (VL CDR3).

The amino acid substitutions may, for example, be conservative amino acid substitutions as described elsewhere herein.

Particularly contemplated for embodiments where the VH and/or VL region sequences differ from those of antibody C, are antibodies where the VH and/or VL region amino acid sequences differ from those of antibody C only in one or more framework region (FR) amino acid sequences. In such embodiments, the antibody retains the VH CDR sequences set forth in SEQ ID NO:2, SEQ ID NO:3, SEQ ID NO:4 and the VL CDR sequences set forth in SEQ ID NO:6, AAS and SEQ ID NO:7.

Accordingly, in some embodiments the antigen-binding region may comprise a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, and a VL region comprising a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7, wherein the VH region has at least 80% identity to SEQ ID NO:1 and the VL region has at least 80% identity to SEQ ID NO:5.

In one specific embodiment, the antibody comprises

- a VH region comprising SEQ ID NO:1 or an amino acid sequence having at least 80% sequence identity, such as 90%, or 95%, or 97%, or 98%, or 99%, to SEQ ID NO:1; and/or
- a VL region comprising SEQ ID NO:5 or an amino acid sequence having at least 80% sequence identity, such as 90%, or 95%, or 97%, or 98%, or 99%, to SEQ ID NO:5.

In another specific embodiment, in the antibody,

- the VH differs from SEQ ID NO:1 by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues; and/or

- the VL differs from SEQ ID NO:5 by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues.

In a specific embodiment, the antibody comprises

- 5 - a heavy chain comprising a VH region comprising SEQ ID NO:1 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, wherein the amino acid residue numbering is according to the EU index, and
- a light chain comprising a VL comprising SEQ ID NO:5.

10 In another specific embodiment, the antibody is a full-length bivalent antibody comprising, consisting, or consisting essentially of two heavy chains and two light chains, wherein

- each heavy chain comprises a VH region and a CH region, wherein the VH region comprises SEQ ID NO:1 and the CH region comprises SEQ ID NO:24 or SEQ ID NO:46, and
- 15 - each light chain comprises a VL region and a CL region, wherein the VL region comprises SEQ ID NO:5 and the CL region comprises SEQ ID NO:37.

Fc region and CH region

20 Mutations in amino acid residues at positions corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index, can improve the ability of an antibody to induce CDC (see, e.g., Example 3). Without being bound by theory, it is believed that by substituting one or more amino acid(s) in these positions, oligomerization of the antibody can be stimulated, thereby modulating effector functions so as to, e.g., increase C1q binding, complement activation, CDC, ADCP, internalization or other relevant function(s) that may provide in vivo efficacy.

25 A pharmaceutical composition of the present invention comprises an antibody comprising an Fc region or a human IgG1 CH region comprising a mutation in one or more of E430, E345 and S440, wherein the amino acid residues are numbered according to the EU index. In the following, reference to mutation(s) in the Fc region may similarly apply to mutation(s) in the human IgG1 CH region, and vice versa.

As described herein, the position of an amino acid to be mutated in the Fc or human IgG1 CH region can be given in relation to (i.e., "corresponding to") its position in a naturally occurring (wild-type) human IgG1 heavy chain, when numbered according to the EU index. So, if the Fc region already contains one or more mutations and/or if the Fc region is, for example, an

5 IgG2, IgG3 or IgG4 Fc region, the position of the amino acid corresponding to an amino acid residue such as, e.g., E430 in a human IgG1 heavy chain numbered according to the EU index can be determined by alignment. Specifically, the Fc region is aligned with a wild-type human IgG1 heavy chain sequence so as to identify the residue in the position corresponding to E430 in the human IgG1 heavy chain sequence. Any wild-type human IgG1 constant

10 region amino acid sequence can be useful for this purpose, including any one of the different human IgG1 allotypes set forth in **Table 1**. This is illustrated in **Figure 1**, which shows an alignment between two different human IgG1 allotypes - IgG1m(f) and IgG1m(a) - and wild-type human IgG2, IgG3 and IgG4, specifically of the segments corresponding to residues P247 to K447 in a human IgG1 heavy chain, wherein the amino acid residues are numbered

15 according to the EU index.

Accordingly, in the remaining paragraphs of this section and elsewhere herein, unless otherwise specified or contradicted by context, the amino acid positions referred to are those corresponding to amino acid residues in a wild-type human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index.

20 In separate and specific embodiments, the Fc region and/or the human IgG1 CH region comprises a mutation in only one of E430, E345 and S440; in both E430 and E345; in both E430 and S440; in both E345 and S440; or in all of E430, E345 and S440. In some embodiments, the Fc region and/or the human IgG1 CH region comprises a mutation in only one of E430, E345 and S440; in both E430 and E345; in both E430 and S440; in both E345

25 and S440; or in all of E430, E345 and S440, with the proviso that any mutation in S440 is S440W or S440Y. In other separate and specific embodiments, the mutation is an amino acid substitution. In one embodiment the mutation is an amino acid substitution in only one of E430X, E345X and S440X; in both E430X and E345X; in both E430X and S440X; in both E345X and S440X; or in all of E430X, E345X and S440X, preferably with the proviso that any

30 mutation in S440X is S440Y or S440W. More preferably, the E430X, E345X and S440X mutations are separately selected from E430G, E345K, E430S, E430F, E430T, E345Q, E345R, E345Y, S440Y and S440W.

In some embodiments, in the antibody, the mutation in the one or more amino acid residues is selected from the group consisting of E430G, E345K, E430S, E430F, E430T, E345Q, E345R,

35 E345Y, S440Y and S440W.

In one embodiment, in the antibody, the mutation in the one or more amino acid residues is selected from the group corresponding to E430G, E345K, E430S and E345Q.

In one embodiment, the mutation is in an amino acid residue corresponding to E430, such as an amino acid substitution, E430X, e.g., selected from those corresponding to E430G, E430S, E430F, or E430T. In one preferred embodiment, the mutation in the one or more amino acid residues comprises E430G. In another preferred embodiment, the mutation in the one or more amino acid residues comprises E430S, optionally wherein no mutations are made in the amino acid residues corresponding to E345 and S440. In a particular embodiment, the mutation in the one or more amino acid residues comprises or consists of E430G. In a particularly preferred embodiment, the mutation in the one or more amino acid residue consists of E430G, i.e., no mutations are made in the amino acid residues corresponding to E345 and S440.

In one embodiment, the mutation is in an amino acid residue corresponding to E345, such as an amino acid substitution, E345X, e.g., selected from those corresponding to E345K, E345Q, E345R and E345Y. In one preferred embodiment, the mutation in the one or more amino acid residues comprises E345K. In another preferred embodiment, the mutation in the one or more amino acid residues comprises E345Q, optionally wherein no mutations are made in the amino acid residues corresponding to E430 and S440. In a particularly preferred embodiment, the mutation in the one or more amino acid residue consists of E345K, i.e., no mutations are made in the amino acid residues corresponding to E430 and S440.

In one embodiment, the mutation is in an amino acid residue corresponding to S440, such as an amino acid substitution, S440X, typically selected from those corresponding to S440Y and S440W. In one preferred embodiment, the mutation in the one or more amino acid residues comprises S440W, optionally wherein no mutations are made in the amino acid residues corresponding to E430 and E345. In one preferred embodiment, the mutation in the one or more amino acid residues comprises S440Y, optionally wherein no mutations are made in the amino acid residues corresponding to E430 and E345.

Preferably, the Fc region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440 in a human IgG1 heavy chain when numbered according to the EU index is, except for any specified mutation(s), a human IgG Fc region selected from the group consisting of a human IgG1, IgG2, IgG3 and IgG4 Fc region. Preferably, the Fc region is, except for any specified mutation(s), a naturally occurring (wild-type) human IgG Fc region, such as a human wild-type IgG1, IgG2, IgG3 or IgG4 Fc region, or a mixed isotype thereof. In one embodiment, the Fc region and/or human IgG1 CH region is, except for any specified mutation(s), a wild-type human IgG1 isotype.

Thus, the Fc region or CH region comprising the mutation in one or more amino acid residues corresponding to E430, E345 and S440 in a human IgG1 heavy chain may, except for any specified mutation(s), be a human IgG1, IgG2, IgG3 or IgG4 isotype, or a mixed isotype thereof. In one embodiment, the Fc region or CH region is, except for any specified
5 mutation(s), a human IgG1 Fc region.

In a specific embodiment, the Fc region and/or human IgG1 CH region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440 in a human IgG1 heavy chain is, except for any specified mutation(s), a human wild-type IgG1m(f), IgG1m(z), IgG1m(a), or IgG1m(x) isotype. In a specific embodiment, the Fc region and/or human IgG1
10 CH region is, except for any specified mutation(s), a human wild-type IgG1 of a mixed allotype, such as IgG1m(za), IgG1m(zax), IgG1m(fa), or the like. In a specific embodiment, the Fc region and/or human IgG1 CH region is, except for any specified mutation(s), a human wild-type IgG1m(za) isotype.

Thus, the Fc region and/or human IgG1 CH region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440 in a human IgG1 heavy chain may, except for any specified mutation(s), be a human IgG1m(f), IgG1m(a), IgG1m(x), IgG1m(z) allotype or a mixed allotype of any two or more thereof.
15

CH region amino acid sequences of specific examples of wild-type human IgG isotypes and IgG1 allotypes are set forth in **Table 1**. In some embodiments, the Fc region comprising the mutation in one or more amino acid residues corresponding to E430, E345 and S440 in a human IgG1 heavy chain comprises, except for any specified mutation(s), the CH2-CH3 or, optionally, the hinge-CH2-CH3 segments of such wild-type CH region amino acid sequences.
20

So, in a specific embodiment, the Fc region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440 in a human IgG1 heavy chain is, except for any specified mutation(s), a human wild-type IgG1 isotype comprising the amino acid residues corresponding to 231-447 in a human IgG1 heavy chain according to the EU numbering. For example, the Fc region may comprise, except for any specified mutation(s), amino acid residues 114 to 330 (direct numbering) of a sequence selected from the group consisting of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID
25 NO:23. In a specific embodiment, the Fc region is, except for any specified mutation(s), a human wild-type IgG1 isotype comprising the amino acid residues corresponding to 216-447 in a human IgG1 heavy chain according to the EU numbering. For example, the Fc region may comprise, except for any specified mutation(s), amino acid residues 99 to 330 (direct numbering) of a sequence selected from the group consisting of SEQ ID NO:19, SEQ ID
30 NO:20, SEQ ID NO:21, SEQ ID NO:22 and SEQ ID NO:23. As described elsewhere herein for
35

production of therapeutic antibodies, the C-terminal amino acid K447 may sometimes be deleted (i.e., absent) or removed. Hence the Fc region may comprise, except for any specified mutation(s), amino acid residues 114 to 329 (direct numbering) or amino acid residues 99 to 329 (direct numbering) of SEQ ID NO: 45.

- 5 In a specific embodiment, the Fc region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440 in a human IgG1 heavy chain is, except for any specified mutation(s), a human wild-type IgG1 isotype comprising the amino acid residues corresponding to 231-447 or 231-446 in a human IgG1 heavy chain according to the EU numbering. For example, the Fc region may comprise amino acid residues 114 to 330
10 (direct numbering) of a sequence selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32 and SEQ ID NO:33. In another embodiment, the Fc region may comprise amino acid residues 114 to 329 (direct numbering) of a sequence selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID
15 NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33 and SEQ ID NO: 46. In yet another embodiment the Fc region may comprise amino acid residues 114 to 329 (direct numbering) of SEQ ID NO: 46.

- In a specific embodiment, the Fc region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440 in a human IgG1 heavy chain is, except for
20 any specified mutation(s), a human wild-type IgG1 isotype comprising the amino acid residues corresponding to 216-447 or 216-446 in a human IgG1 heavy chain according to the EU numbering. For example, the Fc region may comprise amino acid residues 99 to 330 (direct numbering) of a sequence selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ
25 ID NO:31, SEQ ID NO:32 and SEQ ID NO:33. In another embodiment, the Fc region may comprise amino acid residues 99 to 329 (direct numbering) of a sequence selected from the group consisting of SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33 and SEQ ID NO:46. In yet another embodiment the Fc region may comprise amino acid residues
30 99 to 329 (direct numbering) of SEQ ID NO: 46.

So, the present invention can be applied to antibody molecules having a human IgG1 heavy chain, such as a human IgG1 heavy chain comprising a human IgG1 CH region amino acid sequence comprising SEQ ID NO:19 (IgGm(za)). Thus, the human IgG1 CH region may comprise, except for any specified mutation(s), the sequence of SEQ ID NO:19.

The present invention can also be applied to antibody molecules having a human IgG1 heavy chain, such as a human IgG1 heavy chain comprising a human IgG1 CH region amino acid sequence comprising SEQ ID NO:20 (IgGm(f)) or SEQ ID NO: 45. Thus, the human IgG1 CH region may comprise, except for any specified mutation(s), the sequence of SEQ ID NO:20.

- 5 In another embodiment the human IgG1 CH region may comprise, except for any specified mutation(s), the sequence of SEQ ID NO: 45.

The present invention can also be applied to antibody molecules having a human IgG1 heavy chain, such as a human IgG1 heavy chain comprising a human IgG1 CH region amino acid sequence comprising SEQ ID NO:21 (IgGm(z)). Thus, the human IgG1 CH region may

10 comprise, except for any specified mutation(s), the sequence of SEQ ID NO:21.

The present invention can also be applied to antibody molecules having a human IgG1 heavy chain, such as a human IgG1 heavy chain comprising a human IgG1 CH region amino acid sequence comprising, SEQ ID NO:22 (IgGm(a)). Thus, the human IgG1 CH region may comprise, except for any specified mutation(s), the sequence of SEQ ID NO:22.

- 15 The present invention can also be applied to antibody molecules having a human IgG1 heavy chain, such as a human IgG1 heavy chain comprising a human IgG1 CH region amino acid sequence comprising SEQ ID NO:23 (IgG1m(x)). Thus, the human IgG1 CH region may comprise, except for any specified mutation(s), the sequence of SEQ ID NO:23.

- In one embodiment, the human IgG1 CH region comprises, except for any specified
- 20 mutation(s), the sequence of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 or SEQ ID NO: 45.

In other separate and specific embodiments, the human IgG1 CH region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:24 to SEQ ID NO:33 and SEQ ID NO: 46.

- 25 In a specific embodiment, the human IgG1 CH region comprises SEQ ID NO:24 (IgG1m(f)-E430G) or SEQ ID NO:46 (IgG1m(f)-E430G, without K447), optionally wherein the light chain comprises a CL comprising SEQ ID NO:37.

In a specific embodiment, the antibody is a monospecific antibody comprising two HCs that are identical in amino acid sequence and two LCs that are identical in amino acid sequence.

- 30 However, Fc regions or human IgG1 CH regions comprising one or more further mutations, i.e., mutations in one or more other amino acid residues other than those corresponding to

E430, E345 and S440 in a human IgG1 heavy chain when numbered according to the EU index, are also contemplated for the antibodies disclosed herein. So, in one embodiment, the Fc region or CH region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440 comprises also one or more further mutations in amino acid residues other than those corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index. Also or alternatively, the Fc region may be a mixed isotype, e.g., where different CH regions derive from different IgG isotypes. Accordingly, as described in more detail below, in addition to the mutation in one or more amino acid residues corresponding to E430, E345 and S440, the Fc region may comprise one or more further mutations as compared to a wild-type (naturally occurring) or mixed isotype human IgG Fc region.

In one embodiment, the Fc region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440, is a human IgG Fc region which comprises one or more further mutations as compared to a wild-type human IgG1, IgG2, IgG3 and IgG4 Fc region, e.g., as set forth in one of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36. Expressed in an alternative manner, the Fc region comprising a mutation in one or more amino acid residues corresponding to E430, E345 and S440 may differ also in one or more further mutations from a wild-type human IgG1, IgG2, IgG3 and IgG4 Fc region, e.g., as set forth in one of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36. For example, in addition to the mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440, the Fc region may differ from the wild-type Fc region by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues.

For example, the C-terminal amino acid Lys (K) at position 447 (Eu numbering) may be absent. Some host cells which are used for production of an antibody may contain enzymes capable of removing the Lys at position 447, and such removal may not be homogenous. Therapeutic antibodies may therefore be produced without the C-terminal Lys (K) to increase the homogeneity of the product. Methods for producing antibodies without the C-terminal Lys (K) are well-known to a person skilled in the art and include genetic engineering of the nucleic acid expressing said antibody, enzymatic methods and use of specific host cells. Thus, for example the Fc region may comprise, except for the mutation in one or more amino acid residues corresponding to E430, E345 and S440, the sequence as set forth in SEQ ID NO: 45, so that the antibody is expressed from recombinant nucleic acids that encodes a heavy chain lacking the K447 residue.

Preferably, any such one or more further mutations do not reduce the ability of the antibody as disclosed herein, i.e., an antibody comprising a mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain, to induce CDC and/or ADCC. More preferably, any such one or more further mutations do not reduce the ability of the antibody to induce CDC. That is, the antibody comprising the Fc region comprising one or more further mutations does not have a reduced CDC and/or ADCC as compared to the antibody without the one or more further mutations. Most preferably, any such one or more further mutations do not reduce the ability of the antibody to induce either one of CDC and ADCC. Candidates for the one or more further mutations can, for example, be tested in CDC or ADCC assays, e.g., as disclosed herein, such as in Examples 3 and 4. For example, the CDC of an antibody as described herein, e.g., IgG1-C-E430G, can be tested in the assay of Example 3 or an assay as described in the next section (or a similar assay) with and without specific candidates for one or more further mutations, so as to ascertain the effect of the candidate further mutation(s) on the ability of the antibody to induce CDC. Likewise, the ADCC of an antibody as described herein, e.g., IgG1-C- E430G, can be tested in the assay of Example 4 or an assay as described in the next section (or a similar assay) with and without a specific candidate for a further mutation so as to ascertain the effect of the candidate further mutation on the ability on the antibody to induce ADCC.

Preferably, in an antibody comprising two HCs and two LCs, the Fc regions in the first and second HC are identical in amino acid sequence such that the Fc region, in dimerized form, is a homodimer.

However, in some embodiments, in an antibody comprising two HCs and two LCs, the Fc region in the first HC may differ in one or more amino acids from the Fc region in the second HC, such that the Fc region, in dimerized form, is a heterodimer. For example, the mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index, may only be present in one of the Fc regions. Accordingly, in some embodiments, one Fc region may be SEQ ID NO:45 or a human wild-type IgG Fc region selected from SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23, SEQ ID NO:34, SEQ ID NO:35 and SEQ ID NO:36 while the other Fc region may be identical except for a mutation in said one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain.

In one embodiment, the antibody according to any aspect or embodiment herein is, except for any specified mutation(s), a human antibody.

In one embodiment, the antibody according to any aspect or embodiment herein is, except for any specified mutation(s), a full-length antibody, such as a human full-length antibody.

In one embodiment, the antibody according to any aspect or embodiment herein is, except for any specified mutation(s), a bivalent antibody, such as a human bivalent antibody, such as a human bivalent full-length antibody.

In one embodiment, the antibody according to any aspect or embodiment herein is, except for any specified mutation(s), a monoclonal antibody, such as a human monoclonal antibody, such as a human bivalent monoclonal antibody, such as a human bivalent full-length monoclonal antibody.

10 In a preferred embodiment, the antibody according to any aspect or embodiment herein is, except for any specified mutation(s), an IgG1 antibody, such as a full length IgG1 antibody, such as a human full-length IgG1 antibody, optionally a human monoclonal full-length bivalent IgG1, κ antibody, e.g. a human monoclonal full-length bivalent IgG1m(f), κ antibody.

15 An antibody according to the present invention is advantageously in a bivalent monospecific format, comprising two antigen-binding regions binding to the same epitope. However, bispecific formats where one of the antigen-binding regions binds to a different epitope are also contemplated. So, the antibody according to any aspect or embodiment herein can, unless contradicted by context, be either a monospecific antibody or a bispecific antibody.

20 In one embodiment, the antibody according to any aspect or embodiment herein is a monospecific antibody, such as a human monospecific antibody, such as a human full-length monospecific antibody, such as a human full-length monospecific bivalent monoclonal antibody. Optionally, except for any specified mutation(s), the antibody may be a human full-length monospecific bivalent IgG1, κ monoclonal antibody, e.g., a human full-length monospecific bivalent IgG1m(f), κ monoclonal antibody.

25 In another embodiment, the antibody according to any aspect or embodiment herein is a multi-specific antibody, such as a full-length bispecific bivalent antibody, such as a human full-length bispecific bivalent antibody, such as a human full-length bispecific bivalent monoclonal antibody. Optionally, except for any specified mutation(s), the antibody may be a human full-length bispecific bivalent IgG1, κ monoclonal antibody, e.g., a human full-length bispecific bivalent IgG1m(f), κ monoclonal antibody.

In a particular embodiment, the pharmaceutical composition according to any aspect or embodiment herein comprises an antibody comprising, consisting or consisting essentially of

two identical heavy chain (HC) amino acid sequences and two identical light chain (LC) amino acid sequences, wherein each HC amino acid sequence comprises a VH region comprising SEQ ID NO:1 and a CH region comprising SEQ ID NO:46, and wherein each LC amino acid sequence comprises a VL region comprising SEQ ID NO:5 and a CL region comprising SEQ ID NO:37.

Modulation of functions

The antibody according to any aspect or embodiment herein can bind to human CD38 and typically induce one or more, preferably all, of CDC, ADCC, ADCP, apoptosis in the presence but not absence of an Fc-cross-linking agent, trogocytosis, or any combination thereof, of target cells expressing human CD38, typically in the presence of complement and effector cells. The antibody according to any aspect or embodiment herein may also typically modulate the enzyme activity of CD38.

Accordingly, in one embodiment, the antibody according to any aspect or embodiment herein may induce one or more of CDC, ADCC, ADCP, apoptosis in the presence but not absence of an Fc-cross-linking agent, trogocytosis, and modulate the enzyme activity of CD38, or any combination thereof.

In one embodiment, the antibody is capable of binding to His-tagged human CD38, as specified in SEQ ID NO: 39.

In one embodiment, the antibody has an inhibitory effect on the cyclase activity of human CD38. For example, the antibody may inhibit the cyclase activity of human CD38 by at least about 40%, such as at least about 50%, such as at least about 60%. The inhibition of cyclase activity can be determined by an assay comprising the steps of:

- a) seeding 200,000 Daudi or Wien133 cells in 100 μ L 20 mM Tris-HCL per well; or seeding 0.6 μ g/mL His-tagged soluble human CD38 (SEQ ID NO:39) in 100 μ L 20 mM Tris-HCL per well in a multi-well plate;
- b) adding 1 μ g/mL CD38 antibody and 80 μ M NGD to each well;
- c) measuring fluorescence until a plateau is reached (e.g.; 5, 10 or 30 minutes); and
- d) determining the percentage inhibition as compared to a control, such as a well incubated with an isotype control antibody.

In one embodiment, the antibody induces apoptosis in the presence, but not in the absence, of an Fc-cross-linking antibody.

In one embodiment, the antibody induces CDC, ADCC, antibody-dependent cell-phagocytosis (ADCP), trogocytosis, or any combination thereof, of cells expressing human CD38.

In one embodiment, the antibody induces CDC of cells expressing human CD38. For example, the antibody may induce CDC against Daudi cells (ATCC No. CCL-213) or Ramos cells (ATCC No. CRL-1596) resulting in a maximum lysis at least 50%, such as at least 60%, such as at least 70% higher than that obtained with a reference antibody differing only in the absence of the one or more mutations in the Fc region. The CDC can be determined by an assay comprising the steps of:

- 10 a) plating 100,000 CD38-expressing cells in 40 μ L culture medium supplemented with 0.2% BSA per well in a multi-well plate;
- b) preincubating cells for 20 minutes with 40 μ L of serially diluted CD38 antibody (0.0002-10 μ g/mL);
- c) incubating each well for 45 minutes at 37°C with 20 percent of pooled normal human serum;
- 15 d) adding a viability dye and measuring the percentage of cell lysis on a flow cytometer; and
- e) determining the maximum lysis using non-linear regression.

More detailed embodiments with respect to modulation of function are described below.

Complement-dependent cytotoxicity (CDC):

- 20 In one embodiment, the antibody as disclosed herein induces CDC. In particular, the antibody may mediate an increased CDC when bound to CD38 on, for example, the surface of a CD38-expressing cell or cell-membrane, as compared to a control. The control can be, for example, a reference antibody with amino acid sequences (typically heavy- and light chain amino acid sequences) identical to the antibody except for the mutation in one or more amino acid
- 25 residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain. Alternatively, the control can be a reference antibody with amino acid sequences (typically heavy- and light chain amino acid sequences) identical to the antibody except for different VH and VL sequences. Such a reference antibody could, for example, instead have the VH and VL sequences of antibody B or A, as shown in Table 1. Preferably,
- 30 the VH and VL sequences of the reference antibody are those of antibody B. Alternatively, the reference antibody may be an antibody binding the same target but with different amino acid sequences. Alternatively, the control may be an isotype control antibody, e.g., such that the VH and VL sequences are those of antibody b12 as shown in Table 1.

Accordingly, in one embodiment, the antibody according to any aspect or embodiment disclosed herein induces a higher CDC against CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody C, i.e., SEQ ID NO:1 and SEQ ID NO:5, respectively, and CH and CL region sequences identical to the antibody except for the one or more mutations in E430, E345 and/or S440.

In another embodiment, the antibody according to any aspect or embodiment disclosed herein induces a higher CDC against CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody C, i.e., SEQ ID NO:1 and SEQ ID NO:5, respectively, and the CH and CL region sequences of SEQ ID NO:20 (IgGm(f)) and SEQ ID NO:37 (kappa), respectively.

In another embodiment, the antibody according to any aspect or embodiment disclosed herein induces a higher CDC against CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody B, i.e., SEQ ID NO:8 and SEQ ID NO:9, respectively, and CH and CL region sequences identical to the antibody.

In another embodiment, the antibody according to any aspect or embodiment disclosed herein induces a higher CDC against CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody A, i.e., SEQ ID NO:10 and SEQ ID NO:11, respectively, and CH and CL region sequences identical to the antibody.

In another embodiment, the antibody according to any aspect or embodiment disclosed herein induces a higher CDC against CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody b12, i.e., SEQ ID NO:12 and SEQ ID NO:16, respectively, and CH and CL region sequences identical to the antibody.

In one specific embodiment, the CDC response is described as maximum lysis, where a higher maximum lysis reflects an increased CDC. In one specific embodiment, the CDC response is described as EC50 (the concentration at which half maximal lysis is observed), where a lower EC50 indicates an increased CDC. In one specific embodiment, the CD38-expressing target cells are tumor cells, such as lymphoma cells. Non-limiting examples of lymphoma target cells include (indicating, within parentheses, a commercial source):

- Daudi cells (ATCC CCL-213);

- Ramos cells (ATCC CRL-1596);
- REH cells (DSMZ ACC 22);
- Wien-133 cells (BioAnaLab, Oxford, U.K.);
- RS4;11 cells (DSMZ ACC 508);
- 5 - NALM-16 (DSMZ ACC 680);
- U266 (ATCC TIB-196);
- RC-K8 (DSMZ ACC 561);
- SU-DHL-8;
- Oci-Ly-7;
- 10 - Oci-Ly-19;
- Oci-Ly-18;
- Raji;
- DOHH-2;
- SU-DHL-4;
- 15 - WSU-DLCL-2;
- Z-138;
- JVM-13;
- Jeko-1;
- 697;
- 20 - Granta 519;

- DB;
- Pfeiffer.

The CD38-expressing target cells may also be an AML cell, such as one selected from the group consisting of but not limited to: THP1, monomac6, Oci-AML3, KG-1, ML2, U937, Nomo-
5 1, AML-193, MEGAL, MOLM13, HL-60, Oci-M1.

The CD38-expressing target cells may also be an MM cell, such as one selected from the group consisting of but not limited to: LP-1, NCI-H929, OPM-2 and ARH77.

In another specific embodiment, the CD38-expressing target cells are tumor cells, such as lymphoma cells, leukemia cells or myeloma cells, wherein the approximate average number
10 of CD38 molecules per cell is in one of the following ranges, optionally when determined as described in Example 1:

- 150,000-250,000, such as about 200,000;
- 200,000-300,000, such as about 260,000;
- 80,000-180,000, such as about 130,000;
- 15 - 50,000-150,000, such as about 100,000;
- 40,000-120,000, such as about 80,000;
- 30,000-70,000, such as about 50,000;
- 10,000-20,000, such as about 15,000;
- 5,000-15,000, such as about 10,000.

20 In one embodiment, the antibody according to any aspect or embodiment as disclosed here induces an increased CDC against CD38-expressing target cells as compared to a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody B, i.e., SEQ ID NO:8 and SEQ ID NO:9, respectively, and CH and CL region sequences identical to the antibody, wherein the CDC-response is EC50 and the CD38-
25 expressing target cells are selected from NALM-16 (DSMZ ACC 680), U266 (ATCC TIB-196) and RC-K8 (DSMZ ACC 561).

In a preferred embodiment, the antibody according to any aspect or embodiment as disclosed herein induces an increased CDC against CD38-expressing target cells as compared to a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody C, i.e., SEQ ID NO:1 and SEQ ID NO:5, respectively and the CH and CL region sequences of SEQ ID NO:20 (IgGm(f)) and SEQ ID NO:37 (kappa), respectively, wherein the CDC-response is maximum lysis and the CD38-expressing target cells are selected from Daudi cells (ATCC CCL-213) and Ramos cells (ATCC CRL-1596). The antibody may in particular result in at least 50%, such as at least 60% or at least 70% higher maximum lysis than the reference antibody.

Any *in vitro* or *in vivo* method or assay known by the skilled person and suitable for evaluating the ability of an antibody, such as an IgG antibody, to induce CDC against CD38-expressing target cells can be used. Preferably, the assay comprises, in relevant part, the steps of the CDC assay described in Example 3.

A non-limiting example of an assay for determining the maximum lysis of CD38 expressing cells as mediated by a CD38 antibody, or the EC50 value, may comprise the steps of:

- (a) plating about 100,000 CD38-expressing cells in 40 μ L culture medium supplemented with 0.2% BSA per well in a multi-well plate;
 - (b) preincubating cells for 20 minutes with 40 μ L of serially diluted CD38 antibody (0.0002-10 μ g/mL);
 - (c) incubating each well for 45 minutes at 37°C with 20 percent of pooled normal human serum;
 - (d) adding a viability dye and measuring the percentage of cell lysis on a flow cytometer;
 - (e) determining the maximum lysis and/or calculating the EC50 value using non-linear regression.
- Tumor cells suitable for this assay include, without limitation, those listed in Table 2, such as Daudi cells (ATCC CCL-213).

In certain embodiments, the antibody induces CDC against Daudi cells (ATCC No. CCL-213) or Ramos cells (ATCC No. CRL-1596) resulting in a maximum lysis at least 50%, such as at least 60%, such as at least 70% higher than that obtained with a reference antibody differing only in the absence of the mutation in the one or more amino acid residues selected from the

group corresponding to E430, E435 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index. In one embodiment, the reference antibody comprises the VH and VL region sequences of antibody C, i.e., SEQ ID NO:1 and SEQ ID NO:5, respectively and the CH and CL region sequences of SEQ ID NO:20 (IgGm(f)) and SEQ ID NO:37 (kappa), respectively.

Antibody-dependent cell-mediated cytotoxicity (ADCC):

In one embodiment, the antibody according to any aspect or embodiment herein induces ADCC. In some embodiments, the antibody may mediate ADCC when bound to CD38 on, for example, the surface of a CD38-expressing cell or cell membrane. The anti-CD38 antibodies comprising an E430G mutation were found to induce slightly lower levels of ADCC compared to the same antibody without an E430G mutation. The antibody may mediate higher ADCC when bound to CD38 on, for example, the surface of a CD38-expressing cell or cell membrane, than a control, wherein the control can be, for example, a reference antibody with amino acid sequences (typically heavy- and light chain amino acid sequences) identical to the antibody except for different VH and VL sequences. Such a reference antibody could, for example, instead have the VH and VL sequences of antibody B or A, as shown in Table 1. Preferably, the VH and VL sequences of the reference antibody are those of antibody B. Alternatively, the control may be an isotype control antibody, e.g., such that the VH and VL sequences are those of antibody b12 as shown in Table 1.

Accordingly, in one embodiment, the antibody according to any aspect or embodiment disclosed herein, induces a higher ADCC against CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody B, i.e., SEQ ID NO:8 and SEQ ID NO:9, respectively and CH and CL region sequences identical to the antibody. In one specific embodiment, the ADCC response is maximum lysis, where a higher maximum lysis reflects a higher ADCC. In one specific embodiment, the ADCC response evaluated in an assay determining FcγRIIIa binding, where a higher binding indicates a higher ADCC. In one specific embodiment, the CD38-expressing target cells are tumor cells. Non-limiting examples of target cells include Daudi, Wien-133, Granta 519, MEC-2 and the tumor cell lines listed in Table 2.

In one embodiment, the antibody according to any aspect or embodiment disclosed herein induces a higher ADCC against CD38-expressing Daudi cells as compared to a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody B, i.e., SEQ ID NO:8 and SEQ ID NO:9, respectively and CH and CL region sequences identical to the antibody, optionally wherein the ADCC response is maximum lysis or FcγRIIIa binding.

In one embodiment, the antibody according to any aspect or embodiment disclosed herein induces a higher ADCC against CD38-expressing Daudi cells as compared to a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody b12, i.e., SEQ ID NO:12 and SEQ ID NO:16, respectively and CH and CL region sequences identical to the antibody, optionally wherein the ADCC response is maximum lysis or FcγRIIIa binding.

Any *in vitro* or *in vivo* method or assay known by the skilled person and suitable for evaluating the ability of an antibody, such as an IgG antibody, to induce ADCC against CD38-expressing target cells can be used. Preferably, the assay comprises, in relevant part, the steps of the ⁵¹Cr-release antibody-dependent cellular cytotoxicity assay or the ADCC reporter bioassay described in Example 4. Non-limiting examples of assays for determining the ADCC of CD38-expressing cells as mediated by a CD38 antibody may comprise the steps of the ⁵¹Cr-release assay or the reporter assay set out below.

ADCC with ⁵¹Cr release:

- (a) plating about 5,000 ⁵¹Cr labelled CD38-expressing cells (e.g., Daudi cells) in 50 μL culture medium supplemented with 0.2% BSA per well in a multi-well plate;
- (b) preincubating cells for 15 minutes with 50 μL of serially diluted CD38 antibody (0.0002-10 μg/mL);
- (c) incubating each well for 4 hours at 37°C with 500,000 freshly isolated peripheral blood mononuclear cells (PBMCs) per well;
- (d) measuring the amount of ⁵¹Cr release in 75 μL supernatant on a gamma counter;
- (e) calculating the percentage of cell lysis as (cpm sample - cpm spontaneous lysis)/(cpm maximal lysis - cpm spontaneous lysis) wherein cpm is counts per minute.

ADCC with reporter assay:

- (a) plating about 5,000 Daudi cells in 10 μL in multi-well plates suitable for optical readings (e.g., 384-well OptiPlates from PerkinElmer Inc.) in a standard medium (e.g., RPMI 1640) supplemented with 25% low IgG serum;
- (b) incubating each well for 6 hours at 37°C with 10 μL engineered Jurkat cells stably expressing the FcγRIIIa receptor, V158 (high affinity) variant, and an NFAT response

element driving expression of firefly luciferase as effector cells and 10 μ L serially diluted CD38 antibody (0.0002-10 μ g/mL);

(c) incubating each well 5 minutes at RT with 30 μ L Luciferase substrate and measuring luminescence.

5 Antibody-dependent cellular phagocytosis (ADCP):

In one embodiment, the antibody according to any aspect or embodiment herein induces ADCP. In some embodiments, the antibody may mediate ADCP when bound to CD38 on, for example, the surface of a CD38-expressing cell or cell membrane. The antibody may mediate a higher ADCP when bound to CD38 on, for example, the surface of a CD38-expressing cell or cell
10 membrane, than a control wherein the control is an isotype control antibody, e.g., such that the VH and VL sequences are those of antibody b12 as shown in Table 1.

Accordingly, in one embodiment, the antibody according to any aspect or embodiment disclosed herein, induces a higher ADCP against CD38-expressing target cells than a reference antibody, wherein the reference antibody differs from the antibody only in the one
15 or more mutations in E430, E345 and/or S440 in the antibody. In an alternative embodiment, the reference antibody comprises the VH and VL region sequences of antibody b12, i.e., SEQ ID NO:12 and SEQ ID NO:16, respectively and CH and CL region sequences identical to the antibody.

In one specific embodiment, the CD38-expressing target cells are tumor cells, such as
20 myeloma or lymphoma cells. Non-limiting examples of target cells that are tumor cells include those listed in Table 2.

Any *in vitro* or *in vivo* method or assay known by the skilled person and suitable for evaluating the ability of an antibody, such as an IgG antibody, to induce ADCP against CD38-expressing target cells can be used. Preferably, the assay comprises, in relevant part, the
25 steps of the macrophage-based ADCP assay described in Example 5. In particular, the assay for determining the ADCP of CD38-expressing cells as mediated by a CD38 antibody may comprise the steps set out below:

ADCP:

(a) differentiating freshly isolated monocytes to macrophages with 5 days incubation in
30 GM-CSF-containing medium;

- (b) plating about 100,000 macrophages per well in a multi-well plate in dendritic cell medium with GM-CSF;
- (c) adding 20,000 CD38-antibody opsonized CD38-expressing cells (e.g., Daudi cells), labelled with a generic fluorescent membrane dye, per well for 45 minutes at 37°C;
- 5 (d) measuring the percentage of CD14-positive, CD19-negative, membrane-dye-positive macrophages on a flow cytometer.

Apoptosis:

- The antibody according to any aspect or embodiment herein may, in one embodiment, not induce apoptosis in the absence of an Fc-cross-linking agent. In a further embodiment the
- 10 antibody may induce apoptosis in the presence of an Fc-cross-linking agent but not in the absence of an Fc-cross-linking agent.

In one embodiment the Fc-cross-linking agent is an antibody.

In one embodiment apoptosis may be determined as described in Example 6.

Trogocytosis:

- 15 In one embodiment, the antibody as disclosed herein induces trogocytosis, such as trogocytosis of CD38 from donor CD38-expressing cells to acceptor cells. Typical acceptor cells include T and B cells, monocytes/macrophages, dendritic cells, neutrophils, and NK cells. Preferably, the acceptor cells are lymphocytes expressing Fc-gamma- (Fcy)-receptors, such as, e.g., macrophages or PBMCs. In particular, the antibody may mediate an increased
- 20 trogocytosis as compared to a control. The control can be, for example, a reference antibody with amino acid sequences (typically heavy- and light chain amino acid sequences) identical to the antibody except for the one or more mutations in E430, E345 and/or S440 in the antibody. In another embodiment, the control is a reference antibody with amino acid sequences (typically heavy- and light chain amino acid sequences) identical to the antibody
- 25 except for different VH and VL sequences. For example, the control may be an isotype control antibody, e.g., such that the VH and VL sequences are those of antibody b12 as shown in Table 1.

- Suitable assays for evaluating trogocytosis are known in the art and include, for example, the assay in Example 8. Non-limiting examples of assays for determining trogocytosis of CD38
- 30 expressing cells as mediated by a CD38 antibody include the following:

Trogocytosis (Daudi cells):

- (a') differentiating freshly isolated monocytes to macrophage with 5 days GM-CSF;
- (b') plating about 100,000 macrophages per well in dendritic cell medium with GM-CSF;
- (c') adding about 20,000 CD38 antibody-opsonized Daudi cells, labelled with a generic
5 fluorescent membrane dye, per well for 45 minutes at 37°C;
- (d') measuring CD38 expression on Daudi cells on a flow cytometer, wherein a reduction in CD38 on CD38-antibody opsonized Daudi cells as compared to a control indicates trogocytosis.

Trogocytosis (Tregs):

- 10 (a) plating about 500,000 freshly isolated PBMCs per well in cell culture medium O/N at 37°C;
- (b) adding about 100,000, CD38 antibody-opsonized Tregs, labelled with a generic fluorescent intracellular amine dye, per well overnight (O/N) at 37°C; and
- 15 (c) measuring CD38 expression on Tregs on a flow cytometer, wherein a reduction in CD38 on CD38-antibody opsonized Tregs as compared to a control indicates trogocytosis.

In addition to Daudi cells (ATCC CCL-213), tumor cells suitable for the first assay include, without limitation, those listed in Table 2, particularly those with a high CD38 expression. Moreover, suitable CD38-expressing cells for the second assay include, in addition to Tregs,
20 immune cells such as, e.g., NK cells, B cells, T cells and monocytes, as well as tumor cells listed in Table 2, particularly those with a low CD38 expression level.

Accordingly, in one embodiment, the antibody according to any aspect or embodiment disclosed herein induces a higher level of trogocytosis of a CD38-expressing target cell than a reference antibody, wherein the reference antibody comprises the VH and VL region
25 sequences of antibody C, i.e., SEQ ID NO:1 and SEQ ID NO:5, respectively, and CH and CL region sequences identical to the antibody except for the one or more mutations in E430, E345 and/or S440.

In some embodiments, the antibody according to any aspect or embodiment disclosed herein induces a higher level of trogocytosis of CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody B, i.e., SEQ ID NO:8 and SEQ ID NO:9, respectively and CH and CL region sequences identical to the antibody.

In some embodiments, the antibody according to any aspect or embodiment disclosed herein induces a higher level trogocytosis of CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody A, i.e., SEQ ID NO:10 and SEQ ID NO:11, respectively and CH and CL region sequences identical to the antibody.

In some embodiments, the antibody according to any aspect or embodiment disclosed herein induces a higher level trogocytosis of CD38-expressing target cells than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody b12, i.e., SEQ ID NO:12 and SEQ ID NO:16, respectively and CH and CL region sequences identical to the antibody.

Modulation of CD38 enzyme activity:

The antibody according to any aspect or embodiment herein can typically modulate one or more enzyme activities of human CD38. In one embodiment, the antibody as disclosed herein has an inhibitory effect on CD38 cyclase activity, e.g. as compared to a control, e.g., an isotype control antibody such as antibody b12. For example, the antibody may have an inhibitory effect on the cyclase activity of CD38 expressed by a cell, such as a tumor cell, and/or an inhibitory effect on isolated CD38, such as a soluble fragment of CD38 (e.g., SEQ ID NO:39).

Any *in vitro* or *in vivo* method or assay known by the skilled person and suitable for evaluating the ability of an anti-CD38 antibody to inhibit CD38 cyclase activity can be used. Suitable assays for testing CD38 cyclase activity are, for example, described in WO 2006/099875 A1 and WO 2011/154453 A1. Preferably, the method comprises, in relevant part, the steps of the particular assay described in Example 6, testing for cyclase activity using nicotinamide guanine dinucleotide sodium salt (NGD) as a substrate for CD38. NGD, which is non-fluorescent, is cyclized by CD38 to a fluorescent analog of cADPR, cyclic GDP-ribose (see, e.g., Comb, Chem High Throughput Screen. 2003 Jun;6(4):367-79A). A non-limiting example of an assay comprises the following steps for determining the inhibition of CD38 cyclase activity:

- (a) seeding 200,000 Daudi or Wien133 cells in 100 μ L 20 mM Tris-HCL per well; or seeding 0.6 μ g/mL His-tagged soluble CD38 (SEQ ID NO:39) in 100 μ L 20 mM Tris-HCL per well in a multi-well plate;
- (b) adding 1 μ g/mL CD38 antibody and 80 μ M NGD to each well;
- 5 (c) measuring fluorescence until a plateau is reached (e.g.; 5, 10 or 30 minutes); and
- (d) determining the percentage inhibition as compared to a control, such as a well incubated with an isotype control antibody.

In one embodiment, in such an assay, an antibody is capable of inhibiting the cyclase activity of CD38, specifically the maximum percent of NGD conversion, with at least about 40%, such as at least about 50%, such as at least about 60%, such as between about 40% to about 60%, as compared to a control, typically CD38 cyclase activity in the presence of an isotype control antibody. For example, the isotype control antibody may comprise the VH and VL region sequences of antibody b12, i.e., SEQ ID NO:12 and SEQ ID NO:16, respectively, and CH and CL region sequences identical to the antibody. In a specific embodiment, the assay
10 utilizes hisCD38 (SEQ ID NO:39) for determining the cyclase activity.
15

In some embodiments, the antibody according to any aspect or embodiment disclosed herein has an increased (i.e., more effective) inhibition of CD38 cyclase activity as compared to a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody B, i.e., SEQ ID NO:8 and SEQ ID NO:9, respectively and CH and CL region sequences identical to the antibody.
20

In some embodiments, the antibody according to any aspect or embodiment disclosed herein has an increased (i.e., more effective) inhibition of CD38 cyclase activity as compared to a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody A, i.e., SEQ ID NO:10 and SEQ ID NO:11, respectively and CH and CL region sequences identical to the antibody.
25

Moreover, in some embodiments, an antibody as described herein induces apoptosis of CD38-expressing cells in the presence, but not in the absence, of Fc-crosslinking antibodies. These functionalities can both be measured in an assay comprising, in relevant part, the steps of the apoptosis assay described in Example 6. In one embodiment, an apoptosis assay may
30 comprise the steps of:

- (a) plating 100,000 CD38-expressing tumor cells in 100 μ L culture medium supplemented with 0.2% BSA per well;
- (b) incubating each well O/N at 37°C with serially diluted CD38 antibody (0.0002-10 μ g/mL) and 10 μ g/mL goat-anti-human IgG1;
- 5 (c) measuring the percentage of dead cells on a flow cytometer.

Affinity:

Methods for evaluating the affinity of an antibody binding to CD38 are well-known in the art and can be used to evaluate the affinity of an antibody as described herein. A particularly suitable assay for evaluating the binding affinity of an antibody as described herein is the one

10 described in Example 13. For example, the assay of Example 13 can be employed to determine the affinity of an antibody according to any aspect or embodiment disclosed herein in comparison to a reference antibody. In some embodiments, the antibody according to any aspect or embodiment disclosed herein has an increased affinity (i.e., a lower K_D) in binding to human CD38 than the reference antibody. In one embodiment, the reference antibody is

15 IgG1-B. In one embodiment, the reference antibody is IgG1-C. In one embodiment, the reference antibody is IgG1-A.

In some embodiments, the antibody according to any aspect or embodiment disclosed herein has an increased affinity (i.e., a lower K_D) in binding to CD38 than a reference antibody, wherein the reference antibody comprises the VH and VL region sequences of antibody B,

20 i.e., SEQ ID NO:8 and SEQ ID NO:9, respectively and an IgG1 Fc region, i.e., IgG1-B.

In some embodiments, the antibody according to any aspect or embodiment disclosed herein is an antibody which comprises a VH which differs from SEQ ID NO:1 by one or more mutations and/or comprises a VL which differs from SEQ ID NO:5 by one or more mutations, and has an increased affinity (i.e., a lower K_D) in binding to CD38 than a reference antibody,

25 wherein the reference antibody comprises the VH and VL region sequences of antibody C, i.e., SEQ ID NO:1 and SEQ ID NO:5, respectively, and CH and CL region sequences identical to the antibody. In a preferred embodiment, the antibody comprises a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the

30 sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7, so that one or more mutations in the VH and/or VL are located in one or more FR regions.

Antibody production

The antibody as disclosed herein is typically produced in a host cell, such as a recombinant host cell. In most embodiments, the antibody will contain both a heavy and a light chain and the host cell therefore typically expresses both heavy- and light-chain-encoding construct, either on the same or a different vector. The host cell may, for example, comprise a first and second nucleic acid construct stably integrated into the cellular genome, wherein the first encodes the heavy chain and the second encodes the light chain of an antibody as disclosed herein. In another embodiment, the present invention provides a cell comprising a non-integrated nucleic acid, such as a plasmid, cosmid, phagemid, or linear expression element, which comprises a first and second nucleic acid construct as specified above.

Accordingly, the invention provides a pharmaceutical composition wherein the antibody is obtained or obtainable by a method comprising expressing the heavy chain and the light chain in a host cell. The host cell line used to produce the antibody can influence the glycosylation profile of the antibody since different host cells may express a different repertoire of glycosylation enzymes and transporters that contributes to the specificity and heterogeneity in glycosylation (Goh and Ng, Critical Reviews in Biotechnology, 2018;38:851-67).

The recombinant host cell of claim can be, for example, a eukaryotic cell, a prokaryotic cell, or a microbial cell, e.g., a transfectoma. In a particular embodiment the host cell is a eukaryotic cell. Other examples of host cells include yeast, bacterial and plant cells. Preferably, the host cell is a mammalian cell, such as CHO, CHO-S, HEK, HEK293, HEK-293F, Expi293F, PER.C6, NS0 cells, Sp2/0 cells or lymphocytic cells. In a particular embodiment, the host cell is a Chinese Hamster Ovary (CHO) cell.

The host cell can be a cell which is capable of Asn-linked glycosylation of proteins, e.g. a eukaryotic cell, such as a mammalian cell, e.g. a human cell. For example, the host cell can be a non-human cell which is genetically engineered to produce glycoproteins having human-like or human glycosylation. Examples of such cells are genetically-modified *Pichia pastoris* (Hamilton et al., Science 301 (2003) 1244-1246; Potgieter et al., J. Biotechnology 139 (2009) 318-325) and genetically-modified *Lemna minor* (Cox et al., Nature Biotechnology 12 (2006) 1591-1597).

The host cell can also be a cell with altered glycosylation machinery. Such cells have been described in the art and can be used as host cells in which to express antibodies of the invention to thereby produce an antibody with altered glycosylation. See, for example, Shields, R.L. et al. (2002) J. Biol. Chem. 277:26733-26740; Umana et al. (1999) Nat.

Biotech. 17:176-1, as well as EP1176195; WO03/035835; and WO99/54342. Additional methods for generating engineered glycoforms are known in the art, and include but are not limited to those described in Davies et al., 2001, Biotechnol Bioeng 74:288-294; Shields et al, 2002, J Biol Chem 277:26733-26740; Shinkawa et al., 2003, J Biol Chem 278:3466-3473), US6602684, WO00/61739A1; WO01/292246A1; WO02/311140A1; WO 02/30954A1; 5 Potelligent™ technology (Biowa, Inc. Princeton, N.J.); GlycoMAb™ glycosylation engineering technology (GLYCART biotechnology AG, Zurich, Switzerland); US 20030115614; Okazaki et al., 2004, JMB, 336: 1239-49, as well as those described in WO2018/114877 WO2018/114878 and WO2018/114879.

10 The host cell may also be a cell which is not capable of efficiently removing C-terminal lysine residues from antibody heavy chains. For example, Table 2 in Liu et al. (2008) J Pharm Sci 97: 2426 (incorporated herein by reference) lists a number of such antibody production systems, e.g. Sp2/0, NS/0 or transgenic mammary gland (goat), wherein only partial removal of a C-terminal lysine residue is obtained. Alternatively, as described elsewhere 15 herein, the nucleic acid sequence that encodes the heavy chain of the antibody may be lack the codon encoding the C-terminal lysine, e.g., the lysine residue corresponding to K447 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index.

In a particular embodiment, the pharmaceutical composition according to the invention 20 comprises a full-length bivalent antibody comprising, consisting, or consisting essentially of

- a heavy chain comprising a VH and a CH, wherein the VH comprises SEQ ID NO:1 and the CH comprises SEQ ID NO:24 or SEQ ID NO:46, and
- and a light chain comprising a VL and a CL, wherein the VL comprises SEQ ID NO:5 and the CL comprises SEQ ID NO:37,

25 wherein the antibody is produced in Chinese hamster ovary (CHO) cells.

In a particular embodiment, the pharmaceutical composition according to the invention comprises a full-length bivalent antibody comprising, consisting, or consisting essentially of

- a heavy chain comprising a VH and a CH, wherein the VH comprises SEQ ID NO:1 and the CH comprises SEQ ID NO:24 or SEQ ID NO:46, and
- 30 - and a light chain comprising a VL and a CL, wherein the VL comprises SEQ ID NO:5 and the CL comprises SEQ ID NO:37,

wherein the antibody is obtained or obtainable by a method comprising expressing the heavy chain and the light chain in a CHO cell.

Therapeutic applications

The pharmaceutical compositions of the present invention have numerous therapeutic utilities involving the treatment of diseases and disorders involving cells expressing CD38, e.g., tumor cells or immune cells expressing CD38. For example, the pharmaceutical compositions may be administered to cells in culture, e.g., in vitro or ex vivo, or to human subjects, e.g., in vivo, to treat or prevent a variety of disorders and diseases. As used herein, the term "subject" is intended to include human and non-human animals which may benefit or respond to the antibody. Subjects may for instance include human patients having diseases or disorders that may be corrected or ameliorated by modulating CD38 function, such as enzymatic activity, and/or induction of lysis and/or eliminating/reducing the number of CD38 expressing cells and/or reducing the amount of CD38 on the cell membrane. Accordingly, the pharmaceutical compositions may be used to elicit in vivo or in vitro one or more of the following biological activities: CDC of a cell expressing CD38 in the presence of complement; inhibition of CD38 cyclase activity; phagocytosis or ADCC of a cell expressing CD38 in the presence of human effector cells; and trogocytosis of CD38-expressing cells, such as tumor cells or immune cells.

Thus, in one aspect, the present invention relates to the pharmaceutical composition according to the present invention for use as a medicament.

In one aspect, the present invention relates to the use of the pharmaceutical composition according to the present invention in the preparation of a medicament for treating or preventing a disease or disorder.

In one aspect, the present invention relates to the pharmaceutical composition according to the present invention for use in the treatment or prevention of a disease or disorder, such as for use in the treatment or prevention of a disease or disorder involving cells expressing CD38, e.g. for use in treating a disease involving cells expressing CD38.

In one aspect, the present invention relates to a method of treatment of a disease or disorder comprising administering the pharmaceutical composition according to the present invention to a subject in need thereof.

In one aspect, the invention relates to the pharmaceutical composition according to the present invention for use in the treatment or prevention of a disease or disorder.

In one aspect, the invention relates to a method of treating a disease or disorder, comprising administering the pharmaceutical composition according to the invention to a subject in need

thereof, typically in a therapeutically effective amount and/or for a time sufficient to treat the disease or disorder.

In one aspect, the present invention relates to a method of treating a disease or disorder,
5 comprising the steps of

- selecting a subject suffering from the disease or disorder, and
- administering to the subject the pharmaceutical composition of the invention, typically in a therapeutically effective amount and/or for a time sufficient to treat the disease or disorder.

10 In one embodiment, the disease or disorder involving cells expressing CD38 is cancer, i.e. a tumorigenic disorder, such as a disorder characterized by the presence of tumor cells or immune cells expressing CD38 including, for example, hematological cancers such as B cell lymphoma, plasma cell malignancies, T/NK cell lymphoma, myeloid malignancies as well as solid tumor malignancies.

15 In some embodiments, the disease or disorder is a cancer involving tumor cells expressing CD38.

In some embodiments, the disease or disorder is a cancer involving immunosuppressive cells expressing CD38, such as non-cancerous immunosuppressive cells expressing CD38.

In some embodiments, the disease or disorder is a cancer involving both tumor cells and
20 immunosuppressive cells expressing CD38.

In some embodiments, the disease or disorder is a cancer involving immunosuppressive cells expressing CD38 and tumor cells which do not express CD38.

In still other embodiments, the disease or disorder is an inflammatory and/or autoimmune disease or disorder involving cells expressing CD38.

25 In still other embodiments, the disease or disorder is a metabolic disorder involving cells expressing CD38.

The pharmaceutical composition may be administered to a subject or patient by any suitable route and mode, as described elsewhere herein. In a particular embodiment, the pharmaceutical composition is administered by intravenous injection or infusion. The pharmaceutical composition may optionally be diluted with a suitable diluent prior to or in
5 connection with the intravenous infusion or injection.

Hematological cancers:

In one aspect, the disease or disorder is a hematological cancer. Examples of such hematological cancers include B cell lymphomas/leukemias including precursor B cell lymphoblastic leukemia/lymphoma and B cell non-Hodgkin's lymphomas; acute promyelocytic
10 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
15 type, nodal and splenic type), hairy cell leukemia, diffuse large B cell lymphoma (DLBCL), Burkitt's lymphoma, plasmacytoma, plasma cell myeloma, plasma cell leukemia, post-transplant lymphoproliferative disorder, Waldenström's macroglobulinemia, plasma cell leukemias and anaplastic large-cell lymphoma (ALCL).

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

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

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

lesions), angioimmunoblastic T cell lymphoma, peripheral T cell lymphoma unspecified, and anaplastic large cell lymphoma.

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

In some embodiments, the hematological cancer is selected from the group consisting of multiple myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (adults) (AML), mantle cell lymphoma (MCL), follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL). In one embodiment, the ALL is B
10 cell acute lymphoblastic leukemia (B-ALL).

In some embodiments, the cancer is selected from the group consisting of multiple myeloma (MM), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), acute myelogenous leukemia (adults) (AML), acute lymphoblastic leukemia (ALL), and follicular lymphoma (FL).

15 In some embodiments, the hematological cancer is selected from the group consisting of multiple myeloma (MM), B cell lymphoma and acute myelogenous leukemia (AML). Non-limiting examples of B cell lymphoma include, for example, mantle cell lymphoma (MCL), Burkitt's lymphoma, follicular lymphoma (FL) and B cell acute lymphoblastic leukemia (B-ALL).

20 In some embodiments, the hematological cancer is selected from the group consisting of multiple myeloma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (adults) (AML), B cell lymphoma, diffuse large B-cell lymphoma (DLBCL) and myelodysplastic syndrome (MDS). In one embodiment the B cell lymphoma is selected from mantle cell lymphoma, Burkitt's lymphoma and follicular lymphoma (FL). In
25 one embodiment, the ALL is B cell acute lymphoblastic leukemia (B-ALL). The pharmaceutical composition for use in the treatment or prevention of the hematological cancer may, for example, be administered to the subject or patient by intravenous injection or infusion. The pharmaceutical composition may optionally be diluted with a suitable diluent prior to or in connection with the intravenous infusion or injection.

30 In some embodiments, the cancer is multiple myeloma (MM).

In some embodiments, the cancer is a B cell lymphoma.

In some embodiments, the cancer is chronic lymphocytic leukemia (CLL).

In some embodiments, the cancer is mantle cell lymphoma (MCL).

In some embodiments, the cancer is diffuse large B-cell lymphoma (DLBCL).

In some embodiments, the cancer is follicular lymphoma (FL).

5 In some embodiments, the cancer is acute myelogenous leukemia (adults) (AML).

In some embodiments, the cancer is acute lymphoblastic leukemia (ALL).

In some embodiments, the cancer is B cell acute lymphoblastic leukemia (B-ALL).

In some embodiments, the cancer is Burkitt's lymphoma.

In some embodiments, the cancer is myelodysplastic syndrome (MDS).

10 *Solid tumor malignancies:*

In one aspect, the disease or disorder is a cancer comprising a solid tumor. That is, the patient suffering from cancer has a solid tumor.

Example of solid tumors include, but are not limited to, melanoma, lung cancer, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, prostate
15 cancer, castration-resistant prostate cancer, stomach cancer, ovarian cancer, gastric cancer, liver cancer, pancreatic cancer, thyroid cancer, squamous cell carcinoma of the head and neck, carcinoma of the esophagus or gastrointestinal tract, breast cancer, fallopian tube cancer, brain cancer, urethral cancer, genitourinary cancer, endometrial cancer, cervical cancer, lung adenocarcinoma, renal cell carcinoma (RCC) (e.g., a kidney clear cell carcinoma
20 or a kidney papillary cell carcinoma), mesothelioma, nasopharyngeal carcinoma (NPC), a carcinomas of the esophagus or gastrointestinal tract, or a metastatic lesion of anyone thereof.

In one preferred embodiment, the solid tumor is from a cancer that contains immunosuppressive cells, such as Tregs, and that express CD38. T regulatory cells (Tregs)
25 can have high expression of CD38, and Tregs with high CD38 expression are more immune suppressive compared to Tregs with intermediate CD38 expression (Krejci J. et al. Blood 2016 128:384-394). Accordingly, without being limited to theory, the ability of antibody

comprised in the pharmaceutical composition according to the invention to reduce the amount of CD38 expressed on Tregs via trogocytosis particularly allows for treatment of solid tumors in patients where the Tregs express CD38. Tregs express CD38 when CD38 expression on Tregs is statistically significant as compared to a control, e.g. expression
5 detected with anti-CD38 antibody vs expression detected with an isotype control antibody using well known methods. This can be tested, e.g., by taking a biological sample such as a blood sample, bone marrow sample or a tumor biopsy.

So, in one aspect, the invention relates to the pharmaceutical composition comprising the pharmaceutical composition, for use in the treatment or prevention of a solid tumor in a
10 subject comprising Tregs expressing CD38.

In another aspect, the invention relates to a method of treating a solid tumor in a subject, comprising Tregs expressing CD38, the method comprising administering the pharmaceutical composition according to the invention, typically in a therapeutically effective amount and/or for a time sufficient to treat the disease or disorder.

15 In some embodiments, the solid tumor is melanoma.

In some embodiments, the solid tumor is lung cancer.

In some embodiments, the solid tumor is squamous non-small cell lung cancer (NSCLC).

In some embodiments, the solid tumor is non-squamous NSCLC.

In some embodiments, the solid tumor is colorectal cancer.

20 In some embodiments, the solid tumor is prostate cancer.

In some embodiments, the solid tumor is castration-resistant prostate cancer.

In some embodiments, the solid tumor is stomach cancer.

In some embodiments, the solid tumor is ovarian cancer.

In some embodiments, the solid tumor is gastric cancer.

25 In some embodiments, the solid tumor is liver cancer.

In some embodiments, the solid tumor is pancreatic cancer.

In some embodiments, the solid tumor is thyroid cancer.

In some embodiments, the solid tumor is squamous cell carcinoma of the head and neck.

5 In some embodiments, the solid tumor is carcinoma of the esophagus or gastrointestinal tract.

In some embodiments, the solid tumor is breast cancer.

In some embodiments, the solid tumor is fallopian tube cancer.

In some embodiments, the solid tumor is brain cancer.

In some embodiments, the solid tumor is urethral cancer.

10 In some embodiments, the solid tumor is genitourinary cancer.

In some embodiments, the solid tumor is endometrial cancer.

In some embodiments, the solid tumor is cervical cancer.

15 In some embodiments, the tumor cells of the solid tumor lack detectable CD38 expression. The tumor cells of the solid tumor lack detectable CD38 expression when CD38 expression on tumor cells isolated from the solid tumor is statistically insignificant when compared to a control, e.g. expression detected with anti-CD38 antibody vs expression detected with an isotype control antibody using well known methods. This can be tested, e.g., by taking a biological sample such as a biopsy, from the tumor.

20 In some embodiments, the cancer is in a patient comprising T regulatory cells expressing CD38.

In specific embodiments, the pharmaceutical composition is administered in a therapeutically effective amount and/or for a sufficient period of time to treat the cancer.

Metabolic disorder:

In one aspect the disease or the disorder is a metabolic disorder. That is, the patient is suffering from a metabolic disorder.

In some embodiments the metabolic disorder is amyloidosis. Amyloidosis is a vast group of diseases defined by the presence of insoluble protein deposits in tissues. Its diagnosis is based on histological findings. In a further embodiment said amyloidosis may be AL amyloidosis.

Patients:

In some embodiments, the pharmaceutical composition of the present invention may be for use in the treatment of multiple myeloma in a patient newly diagnosed with multiple myeloma.

In some embodiments, the pharmaceutical composition of the present invention may be for use in the treatment or prevention of a disease or disorder in a subject who have received at least one prior therapy for the same disease or disorder with one or more compounds, wherein said one or more compounds are different from the pharmaceutical composition of the present invention. The disease or disorder may be any disease or disorder described herein; such as a cancer, inflammatory and/or autoimmune disease or disorder involving cells expressing CD38, or a metabolic disorder involving cells expressing CD38.

For example, in some embodiments the pharmaceutical composition of the present invention may be for the use in treatment or prevention of a disease or disorder in a subject who has received a prior treatment with a proteasome inhibitor (PI) and/or an immunomodulatory drug (IMiD). Examples of proteasome inhibitors include but are not limited to bortezomib, carfilzomib and ixazomib. Examples of IMiDs include but are not limited to thalidomide, lenalidomide and pomalidomide. In a further embodiment said disease or disorder may be a cancer or a tumor, such as multiple myeloma, mantle cell lymphoma or myelodysplastic syndrome (MDS). Thus, the subject may be a cancer patient, such as a multiple myeloma, mantle cell lymphoma or myelodysplastic syndrome (MDS) patient. In a specific embodiment, the pharmaceutical composition of the present invention is for the use in treatment or prevention of multiple myeloma in a subject or patient who has received a prior treatment with a proteasome inhibitor (PI) and/or an immunomodulatory drug (IMiD). The pharmaceutical composition may, for example, be administered to the subject or patient by intravenous injection or infusion. The pharmaceutical composition may optionally be diluted with a suitable diluent prior to or in connection with the intravenous infusion or injection.

The pharmaceutical composition of the present invention may be for the use in treatment or prevention of a disease or disorder in a subject which have not had any prior treatment with an anti-CD38 antibody. Typically, such a subject or patient is referred to as an anti-CD38 antibody naïve patient. In one embodiment the anti-CD38 antibody is daratumumab; i.e. the subject or patient have not had any prior treatment with daratumumab. Thus, in one embodiment the subject or patient is a daratumumab-naïve subject/patient. The disease or disorder may be a cancer or tumor, or a metabolic disease, such amyloidosis, according to any aspect or embodiment disclosed herein. In a specific embodiment, the pharmaceutical composition of the invention is for the use in treatment or prevention of multiple myeloma (MM) in a daratumumab-naïve subject or patient. In a particular embodiment, the pharmaceutical composition is administered to the subject or patient by intravenous injection or infusion. The pharmaceutical composition may optionally be diluted with a suitable diluent prior to or in connection with the intravenous infusion or injection.

The present invention also provides the pharmaceutical composition for the use of treatment or prevention of a disease or disorder in a subject who have received at least one prior therapy comprising a CD38 antibody.

The present invention also provides the pharmaceutical composition for use in treating cancer patients who have received at least one prior therapy comprising a CD38 antibody. The present invention also provides the pharmaceutical composition for use in treating patients with a metabolic disease, such as amyloidosis, who have received at least one prior therapy comprising a CD38 antibody. Such a prior therapy may have been one or more cycles of a planned treatment program comprising CD38 antibody, such as one or more planned cycles of CD38 antibody as single-agent therapy or in a combination therapy, as well as a sequence of treatments administered in a planned manner. In one embodiment, the prior therapy was CD38 antibody monotherapy. In one embodiment, the prior therapy was a combination therapy comprising a CD38 antibody. For example, the prior therapy may have been CD38 antibody in combination with a proteasome inhibitor (PI) and an immunomodulatory agent. In some embodiments, the CD38 antibody is daratumumab.

In some aspects, the cancer patient may also be one where administration of daratumumab as a monotherapy has a limited effect.

In some aspects, the cancer can be characterized as cancer that is "refractory" or "relapsed" to a prior therapy. In a further embodiment, the prior therapy may comprise one or more of a PI, an IMiD, and a CD38 antibody, e.g. wherein the CD38 antibody is daratumumab. Typically, this indicates that the prior therapy achieved less than a complete response (CR), for example, that the cancer was non-responsive to CD38 antibody mono- or combination

therapy or that the cancer progressed within a predetermined period of time after the end of CD38 antibody therapy. Examples of such combination therapies include, but are not limited to, combination of a CD38 antibody with a PI or an IMiD or a combination of a PI and an IMiD. Similarly, it may indicate that that the prior therapy achieved less than a complete
5 response (CR), for example, that the cancer was non-responsive to a PI, or an IMiD or a combination therapy thereof, or that the cancer progressed within a predetermined period of time after the end of said therapy. The skilled person can determine whether a cancer is refractory to a prior therapy based on what is known in the art, including guidelines available for each cancer.

10 For example, in multiple myeloma, refractory and relapsed disease can be identified according to the guidelines published by Rajkumar, Harousseau et al., on behalf of the International Myeloma Workshop Consensus Panel, *Consensus recommendations for the uniform reporting of clinical trials: report of the International Myeloma Workshop Consensus Panel*, Blood 2011;117:4691-4695.

15 *Refractory* myeloma can be defined as disease that is nonresponsive while on primary or salvage therapy, or progresses within 60 days of last therapy. Nonresponsive disease is defined as either failure to achieve minimal response or development of progressive disease (PD) while on therapy. There may be 2 categories of refractory myeloma: "relapsed-and-refractory myeloma" and "primary refractory myeloma".

20 *Relapsed and refractory* myeloma can be defined as disease that is nonresponsive while on salvage therapy, or progresses within 60 days of last therapy in patients who have achieved minimal response (MR) or better at some point previously before then progressing in their disease course.

Primary refractory myeloma can be defined as disease that is nonresponsive in patients who
25 have never achieved a minimal response or better with any therapy. It includes patients who never achieve MR or better in whom there is no significant change in M protein and no evidence of clinical progression as well as primary refractory, PD where patients meet criteria for true PD. On reporting treatment efficacy for primary refractory patients, the efficacy in these 2 subgroups ("nonresponding-nonprogressive" and "progressive") should be separately
30 specified.

Relapsed myeloma can be defined as previously treated myeloma that progresses and requires the initiation of salvage therapy but does not meet criteria for either "primary refractory myeloma" or "relapsed-and-refractory myeloma" categories.

For details on specific responses (CR, PR etc.) in multiple myeloma and how to test them, see Rajkumar, Harousseau et al., 2011 (*supra*).

Accordingly, in some embodiments, the pharmaceutical composition according to any aspect or embodiment herein is for use in treating a cancer which is refractory to a prior treatment comprising one or more of a PI, an IMiD and a CD38 antibody. In one embodiment the prior treatment comprises a CD38 antibody. In one embodiment the prior treatment comprises a PI and/or an IMiD. In one embodiment the prior treatment comprises two or more of a CD38 antibody and a PI and an IMiD. In a specific embodiment, the cancer is identified as a refractory cancer before the use.

10 In another embodiment, there is provided for a method for treating cancer in a subject, comprising the steps of:

(i) identifying the subject as being refractory to a prior treatment comprising one or more of a PI, an IMiD and a CD38 antibody, and

(ii) administering a therapeutically effective amount of the pharmaceutical composition according to any aspect or embodiment herein to the subject.

In one embodiment the prior treatment comprises a CD38 antibody. In one embodiment the prior treatment comprises a PI and/or an IMiD. In one embodiment the prior treatment comprises two or more of a CD38 antibody, a PI and an IMiD.

In another embodiment, there is provided for a method for treating cancer refractory to a prior treatment comprising one or more of a PI, an IMiD and a CD38 antibody in a subject, comprising administering a therapeutically effective amount of the pharmaceutical composition according to any aspect or embodiment herein to the subject. In one embodiment the prior treatment comprises a CD38 antibody. In one embodiment the prior treatment comprises a PI and/or an IMiD. In one embodiment the prior treatment comprises two or more of a CD38 antibody, a PI and/or an IMiD.

In some embodiments the PI is selected from the group consisting of bortezomib, carfilzomib and ixazomib.

In some embodiments the IMiD is selected from the group consisting of thalidomide, lenalidomide and pomalidomide.

30 In some embodiments, the CD38 antibody is daratumumab.

In some embodiments, the pharmaceutical composition according to any aspect or embodiment herein is for use in treating a cancer which is relapsed after a prior treatment comprising one or more of a PI, an IMiD and a CD38 antibody. In one embodiment the prior treatment comprises a CD38 antibody. In one embodiment the prior treatment comprises a
5 PI and/or an IMiD. In one embodiment the prior treatment comprises two or more of a CD38 antibody, a PI and an IMiD. In a specific embodiment, the cancer is identified as relapsed before the use.

In another embodiment, there is provided for a method for treating cancer in a subject, comprising the steps of:

- 10 (i) identifying the subject as being relapsed after a prior treatment comprising one or more of a PI, an IMiD and a CD38 antibody, and
- (ii) administering a therapeutically effective amount of the pharmaceutical composition according to any aspect or embodiment herein to the subject.

In one embodiment the prior treatment comprises a CD38 antibody. In one embodiment the
15 prior treatment comprises a PI and/or an IMiD. In one embodiment the prior treatment comprises two or more of a CD38 antibody, a PI and an IMiD.

In another embodiment, there is provided for a method for treating cancer relapsed after a prior treatment comprising one or more of a PI, an IMiD and a CD38 antibody in a subject, comprising administering a therapeutically effective amount of the pharmaceutical
20 composition according to any aspect or embodiment herein to the subject. In one embodiment the prior treatment comprises a CD38 antibody. In one embodiment the prior treatment comprises a PI and/or an IMiD. In one embodiment the prior treatment comprises two or more of a CD38 antibody, a PI and an IMiD.

In some embodiments the PI is selected from the group consisting of bortezomib, carfilzomib
25 and ixazomib.

In some embodiments the IMiD is selected from the group consisting of thalidomide, lenalidomide and pomalidomide.

In some embodiments, the CD38 antibody is daratumumab.

In specific embodiments, the pharmaceutical composition according to the present invention is administered in a therapeutically effective amount and/or for a sufficient period of time to treat the refractory or relapsed cancer.

In some embodiments, the refractory or relapsed cancer is a hematological cancer.

- 5 In some embodiments, the refractory or relapsed cancer is selected from the group consisting of multiple myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (adults) (AML), mantle cell lymphoma (MCL), follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL).

- 10 In some embodiments, the refractory or relapsed cancer is selected from the group consisting of multiple myeloma (MM), chronic lymphocytic leukemia (CLL), mantle cell lymphoma (MCL), diffuse large B-cell lymphoma (DLBCL), and follicular lymphoma (FL).

In some embodiments, the refractory or relapsed cancer is multiple myeloma (MM).

In some embodiments, the refractory or relapsed cancer is chronic lymphocytic leukemia (CLL).

- 15 In some embodiments, the refractory or relapsed cancer is mantle cell lymphoma (MCL).

In some embodiments, the refractory or relapsed cancer is diffuse large B-cell lymphoma (DLBCL).

In some embodiments, the refractory or relapsed cancer is follicular lymphoma (FL).

- 20 In a specific embodiment, the pharmaceutical composition according to any aspect or embodiment herein is for use in treating refractory or relapsed multiple myeloma (MM) in a subject or patient after a prior treatment comprising a PI, an IMiD or both. In a particular embodiment, the pharmaceutical composition is administered to the subject or patient by intravenous injection or infusion. The pharmaceutical composition may optionally be diluted with a suitable diluent prior to or in connection with the intravenous infusion or injection.

- 25 In some embodiments, the refractory or relapsed cancer is a solid tumor. In some embodiments, the refractory or relapsed cancer is selected from the group consisting of melanoma, lung cancer, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, prostate cancer, castration-resistant prostate cancer, stomach cancer, ovarian cancer, gastric cancer, liver cancer, pancreatic cancer, thyroid cancer,

squamous cell carcinoma of the head and neck, carcinoma of the esophagus or gastrointestinal tract, breast cancer, fallopian tube cancer, brain cancer, urethral cancer, genitourinary cancer, endometrial cancer, cervical cancer.

In some embodiments, the refractory or relapsed cancer is melanoma.

- 5 In some embodiments, the refractory or relapsed cancer is lung cancer.

In some embodiments, the refractory or relapsed cancer is squamous non-small cell lung cancer (NSCLC).

In some embodiments, the refractory or relapsed cancer is non-squamous NSCLC.

In some embodiments, the refractory or relapsed cancer is colorectal cancer.

- 10 In some embodiments, the refractory or relapsed cancer is prostate cancer.

In some embodiments, the refractory or relapsed cancer is castration-resistant prostate cancer.

In some embodiments, the refractory or relapsed cancer is stomach cancer.

In some embodiments, the refractory or relapsed cancer is ovarian cancer.

- 15 In some embodiments, the refractory or relapsed cancer is gastric cancer.

In some embodiments, the refractory or relapsed cancer is liver cancer.

In some embodiments, the refractory or relapsed cancer is pancreatic cancer.

In some embodiments, the refractory or relapsed cancer is thyroid cancer.

- 20 In some embodiments, the refractory or relapsed cancer is squamous cell carcinoma of the head and neck.

In some embodiments, the refractory or relapsed cancer is carcinoma of the esophagus or gastrointestinal tract.

In some embodiments, the refractory or relapsed cancer is breast cancer.

In some embodiments, the refractory or relapsed cancer is fallopian tube cancer.

In some embodiments, the refractory or relapsed cancer is brain cancer.

In some embodiments, the refractory or relapsed cancer is urethral cancer.

In some embodiments, the refractory or relapsed cancer is genitourinary cancer.

5 In some embodiments, the refractory or relapsed cancer is endometrial cancer.

In some embodiments, the refractory or relapsed cancer is cervical cancer.

Autoimmune and inflammatory diseases and disorders:

In another embodiment of the present invention, the disorder involving cells expressing CD38 is an immune disorder in which CD38 expressing B cells, macrophages, plasma cells,
10 monocytes and T cells are involved, such as an inflammatory and/or autoimmune disease. Examples of immune disorders in which CD38 expressing B cells, plasma cells, monocytes and T cells are involved include autoimmune disorders, such as psoriasis, psoriatic arthritis, dermatitis, systemic scleroderma and sclerosis, inflammatory bowel disease (IBD), Crohn's disease, ulcerative colitis, respiratory distress syndrome, meningitis, encephalitis, uveitis,
15 glomerulonephritis, eczema, asthma, atherosclerosis, leukocyte adhesion deficiency, multiple sclerosis, Raynaud's syndrome, Sjögren's syndrome, juvenile onset diabetes, Reiter's disease, Behçet's disease, immune complex nephritis, IgA nephropathy, IgM polyneuropathies, immune-mediated thrombocytopenias, such as acute idiopathic thrombocytopenic purpura and chronic idiopathic thrombocytopenic purpura, hemolytic anemia, myasthenia gravis,
20 lupus nephritis, systemic lupus erythematosus, rheumatoid arthritis (RA), atopic dermatitis, pemphigus, Graves' disease, Hashimoto's thyroiditis, Wegener's granulomatosis, Omenn's syndrome, chronic renal failure, acute infectious mononucleosis, multiple sclerosis, HIV, and herpes virus associated diseases. Further examples are severe acute respiratory distress syndrome and choreoretinitis. Furthermore, other diseases and disorders are included such as
25 those caused by or mediated by infection of B-cells with virus, such as Epstein-Barr virus (EBV).

In one embodiment, the disorder involving cells expressing CD38 is rheumatoid arthritis.

Further examples of inflammatory, immune and/or autoimmune disorders in which autoantibodies and/or excessive B and T lymphocyte activity are prominent and which may
30 be treated according to the present invention include the following: vasculitides and other

vessel disorders, such as microscopic polyangiitis, Churg-Strauss syndrome, and other ANCA-associated vasculitides, polyarteritis nodosa, essential cryoglobulinaemic vasculitis, cutaneous leukocytoclastic angiitis, Kawasaki disease, Takayasu arteritis, giant cell arthritis, Henoch-Schönlein purpura, primary or isolated cerebral angiitis, erythema nodosum, thrombangiitis obliterans, thrombotic thrombocytopenic purpura (including hemolytic uremic syndrome), and secondary vasculitides, including cutaneous leukocytoclastic vasculitis (e.g., secondary to hepatitis B, hepatitis C, Waldenström's macroglobulinemia, B-cell neoplasias, rheumatoid arthritis, Sjögren's syndrome, or systemic lupus erythematosus); further examples are erythema nodosum, allergic vasculitis, panniculitis, Weber-Christian disease, purpura hyperglobulinaemica, and Buerger's disease; skin disorders, such as contact dermatitis, linear IgA dermatosis, vitiligo, pyoderma gangrenosum, epidermolysis bullosa acquisita, pemphigus vulgaris (including cicatricial pemphigoid and bullous pemphigoid), alopecia areata (including alopecia universalis and alopecia totalis), dermatitis herpetiformis, erythema multiforme, and chronic autoimmune urticaria (including angioneurotic edema and urticarial vasculitis);

immune-mediated cytopenias, such as autoimmune neutropenia, and pure red cell aplasia; connective tissue disorders, such as CNS lupus, discoid lupus erythematosus, CREST syndrome, mixed connective tissue disease, polymyositis/dermatomyositis, inclusion body myositis, secondary amyloidosis, cryoglobulinemia type I and type II, fibromyalgia, phospholipid antibody syndrome, secondary hemophilia, relapsing polychondritis, sarcoidosis, stiff man syndrome, and rheumatic fever; a further example is eosinophil fasciitis; arthritides, such as ankylosing spondylitis, juvenile chronic arthritis, adult Still's disease, and SAPHO syndrome; further examples are sacroileitis, reactive arthritis, Still's disease, and gout; hematologic disorders, such as aplastic anemia, primary hemolytic anemia (including cold agglutinin syndrome), hemolytic anemia secondary to CLL or systemic lupus erythematosus; POEMS syndrome, pernicious anemia, and Waldenström's purpura hyperglobulinaemica; further examples are agranulocytosis, autoimmune neutropenia, Franklin's disease, Seligmann's disease, gamma heavy chain disease, paraneoplastic syndrome secondary to thymoma and lymphomas, an, paraneoplastic syndrome secondary to thymoma and lymphomas, and factor VIII inhibitor formation; endocrinopathies, such as polyendocrinopathy, and Addison's disease; further examples are autoimmune hypoglycemia, autoimmune hypothyroidism, autoimmune insulin syndrome, de Quervain's thyroiditis, and insulin receptor antibody-mediated insulin resistance; hepato-gastrointestinal disorders, such as celiac disease, Whipple's disease, primary biliary cirrhosis, chronic active hepatitis, and primary sclerosing cholangitis; a further example is autoimmune gastritis; nephropathies, such as rapid progressive glomerulonephritis, post-streptococcal nephritis, Goodpasture's syndrome, membranous glomerulonephritis, and cryoglobulinemic nephritis; a further example is minimal change disease; neurological disorders, such as autoimmune neuropathies, mononeuritis multiplex, Lambert-Eaton's myasthenic syndrome, Sydenham's chorea, tabes dorsalis, and Guillain-Barré's syndrome; further examples are

- myelopathy/tropical spastic paraparesis, myasthenia gravis, acute inflammatory demyelinating polyneuropathy, and chronic inflammatory demyelinating polyneuropathy; multiple sclerosis; cardiac and pulmonary disorders, such as COPD, fibrosing alveolitis, bronchiolitis obliterans, allergic aspergillosis, cystic fibrosis, Löffler's syndrome, myocarditis, and pericarditis; further examples are hypersensitivity pneumonitis, and paraneoplastic syndrome secondary to lung cancer; allergic disorders, such as bronchial asthma and hyper-IgE syndrome; a further example is amaurosis fugax; ophthalmologic disorders, such as idiopathic chorioretinitis; infectious diseases, such as parvovirus B infection (including hands-and-socks syndrome); gynecological-obstretical disorders, such as recurrent abortion, recurrent fetal loss, and intrauterine growth retardation; a further example is paraneoplastic syndrome secondary to gynaecological neoplasms; male reproductive disorders, such as paraneoplastic syndrome secondary to testicular neoplasms; and transplantation-derived disorders, such as allograft and xenograft rejection, and graft-versus-host disease.

In one embodiment, the disease or disorder is rheumatoid arthritis.

15 Table 1. Amino acid and nucleic acid sequences

SEQ ID NO:	DESIGNATION	SEQUENCE
1	VH-3003-C	QVQLVQSGAEVKKPGSSVKVSCKAF GGTFSSYA ISWVRQAPG QGLEWMGR IIRFLGIA NYAQKFQGRVTLIADKSTNTAYMELS SLRSEDVAVYYC AGEPGERDPDAVDI WGQGTMTVTVSS
2	VH-3003-C_CDR1	GGTFSSYA
3	VH-3003-C_CDR2	IIRFLGIA
4	VH-3003-C_CDR3	AGEPGERDPDAVDI
5	VL (Kappa)-3003-C	DIQMTQSPSSLSASVGDRTITCRAS QGIRSW LAWYQQKPEK APKSLIY AAS LSQSGVPSRFSGSGSGTDFTLTISSLPEDFA TYYC QQYNSYPLT FGGGTKVEIK
6	VL (Kappa)-3003-C_CDR1	QGIRSW
	VL (Kappa)-3003-C_CDR2	AAS
7	VL (Kappa)-3003-C_CDR3	QQYNSYPLT
8	VH-3003-B	EVQLLESGGGLVQPGGSLRLSCAVS GFTFNSFAM SWVRQAPG KGLEWVSA ISGSGGGT YYADSVKGRFTISRDNKNTLYLQMN SLRAEDTAVYFC AKDKILWFGEFVFDY WGQGTMTVTVSS

9	VL (Kappa) -3003-B	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQ APRLLIYDASNRAITGIPARFSGSGSGTDFTLTISSELPEDFA VYYCQQRSNWPPTEFGQGTKVEIK
10	VH-3003-A	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAFSWVRQAPG QGLEWMGRVIPFLGIANSAQKFQGRVTITADKSTSTAYMDLS SLRSEDATVYYCARRDIAALGPFDDYWGQGLTVTVSS
11	VL (Kappa) -3003-A	DIQMTQSPSSLSASVGDRTITCRASQGISSWLAWYQQKPEK APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISSELPEDFA TYYCQQYNSYPRTTEFGQGTKVEIK
12	VH-gp120-b12	QVQLVQSGAEVKKPGASVKVSCQASGYRFSNFVIHWVRQAPG QRFELMGWVINYNGNKEFSAKFQDRVTFTADTSANTAYMELR SLRSADTAVYYCARVGPYSWDDSPQDNYYMDVWGKGTITVIVS S
13	VH-gp120-b12_CDR1	GYRFSNFV
14	VH-gp120-b12_CDR2	INPYNGNK
15	VH-gp120-b12_CDR3	ARVGPYSWDDSPQDNYYMDV
16	VL-gp120-b12	EIVLTQSPGTLTLSPGERATFSCRSSHSIRSRRAVWYQHKPG QAPRLVIHGVSNRASGISDRFSGSGSGTDFTLTITRVEPEDF ALYYCQVYGASSYTFGQGTKLERK
17	VL-gp120-b12_CDR1	HSIRSRRA
	VL-gp120-b12_CDR2	GVS
18	VL-gp120-b12_CDR3	QVYGASSYT
19	constant region human HC IgG1m(za) (Uniprot entry P01857)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
20	constant region human HC IgG1m(f)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPGK
21	constant region human HC IgG1m(z)	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV

		KGFYP ^S DI ^A VE ^W ES ^N GQ ^P EN ^N YK ^T TP ^P VL ^D SD ^G SFF ^L Y ^S KL ^T VDK ^S R ^W Q ^Q GN ^V F ^S CS ^V M ^H EAL ^H NH ^Y TQ ^K S ^L S ^L SP ^G K
22	constant region human HC IgG1m(a)	ASTK ^G P ^S V ^F FL ^A P ^S SK ^S TSG ^G TAAL ^G CL ^V K ^D Y ^F PE ^P V ^T VS ^W N SGAL ^T SG ^V H ^T FP ^A VL ^Q SS ^G LY ^S LSS ^V V ^T VP ^S SSL ^G TQ ^T Y ^I C ^N VN ^H K ^P S ^N T ^K V ^D K ^R VE ^P K ^S CD ^K TH ^T CP ^P CP ^A PE ^L LG ^G PS ^V FL ^F PP ^K PK ^D TL ^M IS ^R TP ^E VT ^C V ^V VD ^V SH ^E D ^E V ^K FN ^W Y ^V D ^G VE ^V H NA ^K TK ^P REE ^Q YN ^S TY ^R V ^V SV ^L TV ^L H ^Q D ^W LN ^G KEY ^K CK ^V SN ^K A LP ^A PI ^E K ^T ISK ^A G ^Q P ^R EP ^Q V ^T LP ^P SR ^D <u>EL</u> T ^K N ^Q V ^S L ^T CL ^V KGFYP ^S DI ^A VE ^W ES ^N GQ ^P EN ^N YK ^T TP ^P VL ^D SD ^G SFF ^L Y ^S KL ^T VDK ^S R ^W Q ^Q GN ^V F ^S CS ^V M ^H EAL ^H NH ^Y TQ ^K S ^L S ^L SP ^G K
23	constant region human HC IgG1m(x)	ASTK ^G P ^S V ^F FL ^A P ^S SK ^S TSG ^G TAAL ^G CL ^V K ^D Y ^F PE ^P V ^T VS ^W N SGAL ^T SG ^V H ^T FP ^A VL ^Q SS ^G LY ^S LSS ^V V ^T VP ^S SSL ^G TQ ^T Y ^I C ^N VN ^H K ^P S ^N T ^K V ^D K ^R VE ^P K ^S CD ^K TH ^T CP ^P CP ^A PE ^L LG ^G PS ^V FL ^F PP ^K PK ^D TL ^M IS ^R TP ^E VT ^C V ^V VD ^V SH ^E D ^E V ^K FN ^W Y ^V D ^G VE ^V H NA ^K TK ^P REE ^Q YN ^S TY ^R V ^V SV ^L TV ^L H ^Q D ^W LN ^G KEY ^K CK ^V SN ^K A LP ^A PI ^E K ^T ISK ^A G ^Q P ^R EP ^Q V ^T LP ^P SR ^E EM ^T KN ^Q V ^S L ^T CL ^V KGFYP ^S DI ^A VE ^W ES ^N GQ ^P EN ^N YK ^T TP ^P VL ^D SD ^G SFF ^L Y ^S KL ^T VDK ^S R ^W Q ^Q GN ^V F ^S CS ^V M ^H E <u>GL</u> HN ^Y TQ ^K S ^L S ^L SP ^G K
24	constant region human HC IgG1m(f) - E430G	ASTK ^G P ^S V ^F FL ^A P ^S SK ^S TSG ^G TAAL ^G CL ^V K ^D Y ^F PE ^P V ^T VS ^W N SGAL ^T SG ^V H ^T FP ^A VL ^Q SS ^G LY ^S LSS ^V V ^T VP ^S SSL ^G TQ ^T Y ^I C ^N VN ^H K ^P S ^N T ^K V ^D K ^R VE ^P K ^S CD ^K TH ^T CP ^P CP ^A PE ^L LG ^G PS ^V FL ^F PP ^K PK ^D TL ^M IS ^R TP ^E VT ^C V ^V VD ^V SH ^E D ^E V ^K FN ^W Y ^V D ^G VE ^V H NA ^K TK ^P REE ^Q YN ^S TY ^R V ^V SV ^L TV ^L H ^Q D ^W LN ^G KEY ^K CK ^V SN ^K A LP ^A PI ^E K ^T ISK ^A G ^Q P ^R EP ^Q V ^T LP ^P SR ^E EM ^T KN ^Q V ^S L ^T CL ^V KGFYP ^S DI ^A VE ^W ES ^N GQ ^P EN ^N YK ^T TP ^P VL ^D SD ^G SFF ^L Y ^S KL ^T VDK ^S R ^W Q ^Q GN ^V F ^S CS ^V M ^H <u>GA</u> LHN ^Y TQ ^K S ^L S ^L SP ^G K
25	constant region human HC IgG1m(f) - E430S	ASTK ^G P ^S V ^F FL ^A P ^S SK ^S TSG ^G TAAL ^G CL ^V K ^D Y ^F PE ^P V ^T VS ^W N SGAL ^T SG ^V H ^T FP ^A VL ^Q SS ^G LY ^S LSS ^V V ^T VP ^S SSL ^G TQ ^T Y ^I C ^N VN ^H K ^P S ^N T ^K V ^D K ^R VE ^P K ^S CD ^K TH ^T CP ^P CP ^A PE ^L LG ^G PS ^V FL ^F PP ^K PK ^D TL ^M IS ^R TP ^E VT ^C V ^V VD ^V SH ^E D ^E V ^K FN ^W Y ^V D ^G VE ^V H NA ^K TK ^P REE ^Q YN ^S TY ^R V ^V SV ^L TV ^L H ^Q D ^W LN ^G KEY ^K CK ^V SN ^K A LP ^A PI ^E K ^T ISK ^A G ^Q P ^R EP ^Q V ^T LP ^P SR ^E EM ^T KN ^Q V ^S L ^T CL ^V KGFYP ^S DI ^A VE ^W ES ^N GQ ^P EN ^N YK ^T TP ^P VL ^D SD ^G SFF ^L Y ^S KL ^T VDK ^S R ^W Q ^Q GN ^V F ^S CS ^V M ^H <u>S</u> ALHN ^Y TQ ^K S ^L S ^L SP ^G K
26	constant region human HC IgG1m(f) - E430F	ASTK ^G P ^S V ^F FL ^A P ^S SK ^S TSG ^G TAAL ^G CL ^V K ^D Y ^F PE ^P V ^T VS ^W N SGAL ^T SG ^V H ^T FP ^A VL ^Q SS ^G LY ^S LSS ^V V ^T VP ^S SSL ^G TQ ^T Y ^I C ^N VN ^H K ^P S ^N T ^K V ^D K ^R VE ^P K ^S CD ^K TH ^T CP ^P CP ^A PE ^L LG ^G PS ^V FL ^F PP ^K PK ^D TL ^M IS ^R TP ^E VT ^C V ^V VD ^V SH ^E D ^E V ^K FN ^W Y ^V D ^G VE ^V H NA ^K TK ^P REE ^Q YN ^S TY ^R V ^V SV ^L TV ^L H ^Q D ^W LN ^G KEY ^K CK ^V SN ^K A LP ^A PI ^E K ^T ISK ^A G ^Q P ^R EP ^Q V ^T LP ^P SR ^E EM ^T KN ^Q V ^S L ^T CL ^V KGFYP ^S DI ^A VE ^W ES ^N GQ ^P EN ^N YK ^T TP ^P VL ^D SD ^G SFF ^L Y ^S KL ^T VDK ^S R ^W Q ^Q GN ^V F ^S CS ^V M ^H <u>F</u> ALHN ^Y TQ ^K S ^L S ^L SP ^G K
27	constant region human HC IgG1m(f) - E430T	ASTK ^G P ^S V ^F FL ^A P ^S SK ^S TSG ^G TAAL ^G CL ^V K ^D Y ^F PE ^P V ^T VS ^W N SGAL ^T SG ^V H ^T FP ^A VL ^Q SS ^G LY ^S LSS ^V V ^T VP ^S SSL ^G TQ ^T Y ^I C ^N VN ^H K ^P S ^N T ^K V ^D K ^R VE ^P K ^S CD ^K TH ^T CP ^P CP ^A PE ^L LG ^G PS ^V FL ^F PP ^K PK ^D TL ^M IS ^R TP ^E VT ^C V ^V VD ^V SH ^E D ^E V ^K FN ^W Y ^V D ^G VE ^V H NA ^K TK ^P REE ^Q YN ^S TY ^R V ^V SV ^L TV ^L H ^Q D ^W LN ^G KEY ^K CK ^V SN ^K A LP ^A PI ^E K ^T ISK ^A G ^Q P ^R EP ^Q V ^T LP ^P SR ^E EM ^T KN ^Q V ^S L ^T CL ^V KGFYP ^S DI ^A VE ^W ES ^N GQ ^P EN ^N YK ^T TP ^P VL ^D SD ^G SFF ^L Y ^S KL ^T VDK ^S R ^W Q ^Q GN ^V F ^S CS ^V M ^H <u>T</u> ALHN ^Y TQ ^K S ^L S ^L SP ^G K

28	constant region human HC IgG1m(f) - E345K	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPRKQPVYTLPPSREEMTKNQVSLTCLV KGFYPDSIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
29	constant region human HC IgG1m(f) - E345Q	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPRQPVYTLPPSREEMTKNQVSLTCLV KGFYPDSIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
30	constant region human HC IgG1m(f) - E345R	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPRRPQVYTLPPSREEMTKNQVSLTCLV KGFYPDSIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
31	constant region human HC IgG1m(f) - E345Y	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPRYPQVYTLPPSREEMTKNQVSLTCLV KGFYPDSIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
32	constant region human HC IgG1m(f) - S440W	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPDSIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKWL ¹ LSLSPGK
33	constant region human HC IgG1m(f) - S440Y	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICN VNHKPSNTKVDKRVKPKSCDKHTCTPPCPAPPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPDSIAVEWESNGQPENNYKTTPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKY ¹ LSLSPGK
34	constant region human HC IgG2 (Uniprot entry P01859)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSNFGTQTYTCN VDHKPSNTKVDKTKVERKCCVECPAPPVAGPSVFLFPPKPK KDTLMISRTPEVTCVVDVSHEDPEVQFNWYVDGVEVHNAKT KPREEQFNSTFRVSVLTVVHQDWLNGKEYKCKVSNKGLPAP

		IEKTISKTKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFY PSDIAVEWESNGQPENNYKTPPMLDSGDSFFLYSKLTVDKS RWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
35	constant region human HC IgG3 (Uniprot entry P01860)	ASTKGPSVFPLAPCSRSTSGGTAAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYTCN VNHKPSNTKVDKRVELKTPGLDTHTCPRCPEPKSCDTPPPC PRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGP SVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVQFKWYVD GVEVHNAKTKPREEQYNSTFRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKTKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSGDSFFL YSKLTVDKSRWQQGNIFCFSVMHEALHNRTQKSLSLSPGK
36	constant region human HC IgG4 (Uniprot entry P01861)	ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYTCN VDHKPSNTKVDKRVESKYGPCCPSCPAPEFLGGPSVFLFPPK PKDTLMI SRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAK TKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPS SIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTPPVLDSDGDSFFLYSRLTVDK SRWQEGNVFCFSVMHEALHNHYTQKSLSLSPGK
37	constant region human Kappa LC	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWK VDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEEKHKV YACEVTHQGLSSPVTKSFNRGEC
38	Human CD38 (Uniprot entry P28907)	MANCEFSPVSGDKPCCRLSRAQLCLGVSIILVILVVLAVV VPRWRQQWSGPGTTKRFPETVLARCVKYTEIHPEMRHVCQS VWDAFKGAFISKHPCNITEEDYQPLMKLGTQTVPCNKILLWS RIKDLAHQFTQVQVQDMFTLEDTLGLYLADDLTWCGEFNTSKI NYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVHVMNLG SRSKI FDKNSTFGSVEVHNLQPEKVQVLEAWVIHGGREDSD LCQDPTIKELESIISKRNIFQFSCCKNIYRPDKFLQCVKNPDS SCTSEI
39	hisCD38	HHHHHHHRWRQTWSGPGTTKRFPETVLARCVKYTEIHPEMRH VCQSVWDAFKGAFISKHPCNITEEDYQPLMKLGTQTVPCNKI LLWSRIKDLAHQFTQVQVQDMFTLEDTLGLYLADDLTWCGEFN TSKINYQSCPDWRKDCSNNPVSVFWKTVSRRFAEAACDVHVM MLNGSRSKI FDKNSTFGSVEVHNLQPEKVQVLEAWVIHGGRE DSRDLQDPTIKELESIISKRNIFQFSCCKNIYRPDKFLQCVKN PEDSSCTSEI
40	VH CDR1 variants	GGTFX ₁ SYA, wherein X ₁ is S or R
41	VH CDR2 variants	IIX ₁ FLGX ₂ X ₃ , wherein X ₁ is R or V; X ₂ is I or K; and X ₃ is A, T or V, such as A or T
42	VH CDR3 variants	X ₁ GEPGX ₂ RDPDAX ₃ DI, wherein X ₁ is A or T; X ₂ is E, D or A, such as E or D; and X ₃ is V or F
43	VL CDR1	QGIRSW
	VL CDR2	AAS
44	VL CDR3 variants	QQYNX ₁ YPLT, wherein X ₁ is S or N

45	constant region human HC IgG1m(f) without Lys (K) at position 447 according to Eu numbering	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFCSCVMHEALHNHYTQKSLSLSPG
46	constant region human HC IgG1m(f)- E430G, without Lys (K) at position 447 according to Eu numbering	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWN SGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICN VNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVFLF PPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVH NAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKA LPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLV KGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFCSCVMH <u>G</u> ALHNHYTQKSLSLSPG

EXAMPLES

The present invention is further illustrated by the following examples which should not be
5 construed as limiting.

Example 1 – Antibodies and cell-lines

Antibody expression constructs

For the expression of human and humanized antibodies used herein, variable heavy (VH) chain
and variable light (VL) chain sequences were prepared by gene synthesis (GeneArt Gene
10 Synthesis; ThermoFisher Scientific) and cloned in pcDNA3.3 expression vectors (ThermoFisher
Scientific) containing a constant region of a human IgG heavy chain (HC) (constant region
human IgG1m(f) HC: SEQ ID NO:20) and/or the constant region of the human kappa light
chain (LC): SEQ ID NO:37. Desired mutations were introduced by gene synthesis. CD38
antibodies in this application have VH and VL sequences derived from previously described
15 CD38 antibodies IgG1-A (WO 2006/099875 A1, WO 2008/037257 A2, WO 2011/154453 A1;
VH: SEQ ID NO:10; VL: SEQ ID NO:11), IgG1-B (WO 2006/099875 A1, WO 2008/037257 A2,
WO 2011/154453 A1; VH: SEQ ID NO:8; VL: SEQ ID NO:9), and IgG1-C (WO 2011/154453
A1; VH: SEQ ID NO:1; VL: SEQ ID NO:5). The human IgG1 antibody b12, an HIV gp120-
specific antibody was used as a negative control in some experiments (Barbas et al., J Mol Biol.
20 1993 Apr 5;230(3):812-23; VH: SEQ ID NO:12; VL: SEQ ID NO:16).

Transient expression antibody constructs

Plasmid DNA mixtures encoding both heavy and light chains of antibodies were transiently transfected in Expi293F cells (Gibco, Cat No A14635) using 293fectin (Life Technologies) essentially as described by Vink et al. (Vink et al., 2014 Methods 65(1):5-10). Antibody concentrations in the supernatants were measured by absorbance at 280 nm. Antibody-containing supernatants were either directly used in in vitro assays, or antibodies were purified as described below.

Antibody purification and quality assessment

Antibodies were purified by Protein A affinity chromatography. Culture supernatants were filtered over a 0.20 µm dead-end filter and loaded on 5 mL MabSelect SuRe columns (GE Healthcare), washed and eluted with 0.02 M sodium citrate-NaOH, pH 3. The eluates were loaded on a HiPrep Desalting column (GE Healthcare) immediately after purification and the antibodies were buffer exchanged into 12.6 mM NaH₂PO₄, 140 mM NaCl, pH 7.4 buffer (B.Braun or Thermo Fisher). After buffer exchange, samples were sterile filtered over 0.2 µm dead-end filters. Purified proteins were analyzed by a number of bioanalytical assays including capillary electrophoresis on sodium dodecyl sulfate-polyacrylamide gels (CE-SDS) and high-performance size exclusion chromatography (HP-SEC). Concentration was measured by absorbance at 280 nm. Purified antibodies were stored at 2-8°C.

The cell-lines used in the Examples are described in **Table 2** below. The average number of CD38 and CD59-molecules per cell was determined by quantitative flow cytometry (Qifi, DAKO).

Table 2. Overview of cell lines and expression of CD38 and CD59

cell line	tumor type	Catalog	supplier	Estimated ABCs	
				CD38	CD59
SU-DHL-8	DLBCL	ACC 573	DSMZ	415000	31000
Oci-Ly-7	DLBCL	ACC 688	DSMZ	310000	81000
Oci-Ly-19	DLBCL	ACC 528	DSMZ	271000	28000
Ramos	Burkitt	CRL-1596	ATCC	260000	7000
Daudi	Burkitt	CCL-213	ATCC	200000	0
Oci-Ly18	DLBCL	ACC 699	DSMZ	181000	40000
Raji	Burkitt	CCL-86	ATCC	170000	55000
DOHH2	FL	ACC 47	DSMZ	167000	66000

SU-DHL-4	DLBCL	ACC 495	DSMZ	158000	147000
WSU-DLCL2	DLBCL	ACC 575	DSMZ	150000	96000
Z-138	MCL	CRL-3001	ATCC	133000	53000
JVM-13	MCL	CRL-3003	ATCC	130000	254000
REH	B-ALL	ACC 22	DSMZ	130000	not tested
Jeko-1	MCL	ACC 553	DSMZ	108000	31000
Wien133	Burkitt	-	BioAnaLab, UK	100000	0
697	B-ALL	ACC 42	DSMZ	98000	130000
Granta-519	MCL	ACC 342	DSMZ	90000	140000
RS4;11	B-ALL	ACC 508	DSMZ	80-86000	not tested
DB	DLBCL	ACC 539	DSMZ	70000	200000
NALM-16	B-ALL	ACC 680	DSMZ	50000	not tested
JVM-3	CLL	ACC 18	DSMZ	30000	not tested
U266	MM	ACC 9	DSMZ	15000	not tested
RC-K8	DLBCL	ACC 561	DSMZ	10000	not tested
Pfeiffer	DLBCL	CRL-2632	ATCC	0	100000
THP-1	AML	ACC 16	DSMZ	400000	40000
Oci-AML3	AML	ACC 582	DSMZ	200000	40000
monomac6	AML	ACC 124	DSMZ	200000	30000
KG-1	AML	CCL-246	ATCC	180000	100000
ML-2	AML	ACC 15	DSMZ	150000	10000
U937	AML	CRL-1593.2	ATCC	130000	not tested
Nomo-1	AML	ACC 542	DSMZ	110000	30000
MEGAL	AML	ACC 719	DSMZ	100000	110000
AML-193	AML	ACC 549	DSMZ	100000	not tested
MOLM-13	AML	ACC 554	DSMZ	90000	10000
HL-60	AML	CLL-240	ATCC	90000	10000
Oci-M1	AML	ACC 529	DSMZ	0	200000
LP-1	MM	ACC 41	DSMZ	130000	15000
NCI-H929	MM	CRL-9068	ATCC	15000	155000
OPM-2	MM	CRL-9068	ATCC	70000	not tested
ARH77	MM	CRL-1621	ATCC	25000	100000

ABCs = Antibodies Bound per Cell

The origins/sources of the cell lines are as follows:

Cell line:

Daudi

Source:

ATCC; CCL-213

Ramos	ATCC; CRL-1596
Wien-133	BioAnaLab, Oxford, U.K
NALM-16	DSMZ; ACC 680
U266	ATCC; TIB-196
RC-K8	DSMZ; ACC 561

Example 2 – Binding of CD38 antibodies to human and cynomolgus CD38 expressed on the cell surface

Binding to cell surface expressed CD38 on Daudi and NALM16 cells and PBMCs from cynomolgus monkeys, was determined by flow cytometry. Cells, resuspended in RPMI

5 containing 0.2% BSA, were seeded at 100,000 cells/well in polystyrene 96 well round-bottom plates (Greiner bio-one) and centrifuged for 3 minutes at 300xg, 4°C. Serial dilutions (0.005-10 µg/mL final antibody concentration in 3x serial dilutions) of CD38 or control antibodies were added and cells were incubated for 30 minutes at 4°C. Plates were washed/centrifuged

10 twice using FACS buffer (PBS/0.1% BSA/0.01% Na-Azide). Next, cells were incubated for 30 minutes at 4°C with R-Phycoerythrin (PE)-conjugated goat-anti-human IgG F(ab')₂ (Jackson) diluted 1/100 in PBS/0.1% BSA/0.01% Na-Azide or FITC-conjugated goat-anti-human IgG (Southern Biotech) for analysis of cynomolgus PBMCs. Cells were washed/centrifuged twice

15 using FACS buffer, resuspended in FACS buffer and analyzed by determining mean fluorescent intensities using a FACS_Fortessa (BD). Binding curves were generated using non-linear regression (sigmoidal dose-response with variable slope) analyses within GraphPad Prism V6.04 software (GraphPad Software).

Figure 2 shows that CD38 antibodies IgG1-B, IgG1-C and IgG1-A bind dose-dependently to CD38 expressing NALM16 cells. Introduction of the hexamerization-enhancing E430G mutation into these antibodies did not affect the binding.

20 **Figure 3** shows that CD38 antibody IgG1-A-E430G, but not IgG1-B-E430G and IgG1-C-E430G, binds dose-dependently to CD38 expressed on cynomolgus PBMCs (A). The average binding to CD38 expressed on cynomolgus B, T and NK cells is depicted, gated based on FSC and SSC. As a positive control, binding to Daudi cells expressing high copy numbers of human CD38 is also depicted (B).

*Example 3 – Complement-dependent cytotoxicity (CDC) by E430G-mutated CD38 antibodies*CDC on tumor cell lines

Daudi, Wien133, Ramos, NALM16, U266 and RC-K8 cells were resuspended in RPMI containing 0.2% BSA and plated into polystyrene 96-well round-bottom plates (Greiner bio-one) at a density of 1×10^5 cells/well (40 μ L/well). CD38 antibodies and isotype control Abs were serially diluted (0.0002-10 μ g/mL final antibody concentration in 3x serial dilutions) and 40 μ L of diluted Ab was added per well. Cells and Ab were pre-incubated for 20 minutes at room temperature after which, 20 μ L of pooled normal human serum (Sanquin) was added to each well and incubated for another 45 minutes at 37°C. After that, plates were centrifuged (3 minutes, 1200 rpm) and supernatant was discarded. Cell pellets were resuspended in FACS-buffer supplemented with 0.25 μ M topro-3 iodide (Life technologies), and lysis was detected by measuring the percentage of topro-3 iodine-positive cells on a FACS_Fortessa (BD). CDC was depicted as percent lysis. Data shown is N=3 (Daudi and NALM16), N=2 (Wien133 and U266 cells), or N=1 (RC-K8 and Ramos). Isotype control antibodies were only included on Daudi and Wien133 cells.

Figure 4 demonstrates that CD38 antibodies B, C and A without the E430G mutation induce ~85, ~50 and 0 percent lysis of Ramos and Daudi cells. No significant lysis by these CD38 antibodies was seen for any of the other tested cell lines. Introduction of an E430G mutation in these CD38 antibodies resulted in higher CDC activity at significantly lower antibody concentration. All 3 antibodies with the E430G mutation induced up to 100% lysis of Ramos and Daudi cells. Moreover, on cell lines with lower CD38 expression, E430G-mutated CD38 antibodies were able to induce maximum (Wien133) or partial (NALM16 and U266) CDC, whereas CD38 antibodies without E430G-mutation did not induce CDC. These results demonstrate that CD38 Abs with an E430G mutation induce stronger CDC and require less CD38 expression compared to the CD38 antibodies without E430G mutation. In tumor cells with lower CD38 expression levels (NALM-16, RS4;11, and REH), IgG1-C-E430G showed lower EC50 values compared to IgG1-B-E430G.

Table 3. EC50-values of lysis

Some cell lines were tested only once (Ramos, RS4;11, REH)

	Ramos	Daudi	Wien-133	NALM-16	U266	RS4;11	REH
B	0.126	0.183	0.199	-	-	-	-

B-E430G	0.019	0.018	0.013	0.075	-	0.243	0.054
C	0.158	0.250	0.193	-	-	-	-
C-E430G	0.014	0.019	0.015	0.022	0.052	0.056	0.017
A	-	-	-	-	-	-	-
A-E430G	0.133	0.206	0.271	-	-	-	-

The above described CDC assay was repeated with a number of further tumor cell lines derived from B-cell tumors, including DLBCL, Burkitt's lymphoma, FL, MCL, B-ALL, CLL, or MM, and the antibodies IgG1-B, IgG1-B-E430G, IgG1-C-E430G, IgG1-A-E430G and isotype control antibody. The percentage lysis was plotted against the antibody concentration and maximum percent lysis and EC50 values were calculated using Graphpad Prism (GraphPad Software, Inc; version 8.1.0) software and shown in Table 4. The results are also shown in Figure 14.

Figure 14 demonstrates that wild type CD38 mAb IgG1-B induced lysis of high CD38 expressing cell lines; SU-DHL-8, Oci-Ly-7, Oci-Ly-19, Ramos, Daudi, Oci-Ly-18 and Raji, but not for any of the other cell lines that express less CD38 molecules on the membrane. Introduction of an E430G mutation in IgG1-B resulted in higher CDC activity at significantly lower Ab concentration on cell lines that were already sensitive to wild type IgG1-B and resulted in lysis of additional cell lines with lower CD38 copy number that were insensitive to IgG1-B induced CDC (e.g.: DOHH2, SU-DHL-4, WSU-DLCL2, Z-138, JVM-13, REH, Jeko-1, Wien-133, 697, RS4;11, NALM-16 and JVM-3). Some cell lines with very low CD38 expression (RC-K8 and Pfeiffer) or very high CD59 expression (DB and Granta-519) showed no lysis upon exposure to IgG1-B and IgG1-B-E430G. On virtually all cell lines tested, IgG1-C-E430G induced cell lysis at a lower antibody concentration compared to IgG1-B-E430G, whereas IgG1-A-E430G induced lysis at much higher Ab concentrations. This is also reflected by the higher EC50 values for IgG1-A-E430G in Table 4. This demonstrates that E430G mutated CD38 mAbs induce stronger CDC compared to wild type CD38 antibodies and induce CDC on tumor cells with lower CD38 expression levels, in which wild type CD38 antibodies do not induce CDC. Moreover, the potency of E430G-mutated CD38 antibodies to induce CDC may vary between different CD38-targeting antibody clones.

Figure 15 shows a summary of some of the EC50 values depicted in Table 4. EC50 values of CDC induced by antibodies IgG1-B, IgG1-B-E430G and IgG1-C-E430G on 20 different B cell tumor cell lines are shown. Each square, triangle or circle represents a different B cell tumor cell line. EC50 values obtained with AML cell lines were not included because IgG1-B-E430G was not tested on AML cell lines.

CDC by IgG1-C-E430G was also evaluated on a selection of Acute Myeloid Leukemia (AML) cell lines (Figure 16). It was performed as described above for the B cell tumor cell lines with the only difference being the tumor cell line(s).

Figure 16 demonstrates that CDC was induced by IgG1-C-E430G in all CD38 expressing AML cell lines, while no CDC was observed in CD38 negative AML cell lines. CDC by IgG1-C-E430G occurred at much lower EC50 value compared to IgG1-B, while the maximal cell lysis was higher for IgG1-C-E430G compared to IgG1-B (Table 4).

In addition, CDC by IgG1-C-E430G was evaluated on additional Multiple Myeloma (MM) cell lines (Figure 23). Experiments were performed as described above for the B cell tumor cell lines with the only difference being the tumor cell line(s).

Figure 23 demonstrates that IgG1-C-E430G induced up to 100% lysis of LP-1 cells. On NCI-H929 cells, which has lower CD38 expression levels, IgG1-C-E430G induced partial CDC, while no CDC was observed in the 2 MM cell lines OPM-2 and ARH77. In the 2 sensitive MM cell lines, CDC induced by IgG1-C-E430G occurred at a lower EC50 value compared to IgG1-B, while the maximal cell lysis was higher for IgG1-C-E430G compared to IgG1-B in the NCI-H929 cell line (Table 4).

Table 4. Maximum lysis and EC50 values of lysis

cell line	IgG1-C-E430G			IgG1-B			IgG1-B-E430G			N
	EC50 ug/mL	max % lysis	min % lysis	EC50 ug/mL	max % lysis	min % lysis	EC50 ug/mL	max % lysis	min % lysis	
SU-DHL-8	0.009	100.0	35.4	0.040	99.8	22.8	0.009	100.0	31.0	3
Oci-Ly-7	0.012	99.2	21.0	0.138	91.7	18.1	0.027	98.9	19.4	3
Oci-Ly-19	0.031	100.0	23.4	0.091	98.8	24.6	0.032	100.0	27.4	3
Ramos	0.013	99.5	25.0	0.108	94.0	17.1	0.020	99.3	19.4	3
Daudi	0.030	96.5	17.9	0.307	89.5	11.3	0.026	96.7	19.1	4
Oci-Ly18	0.057	92.5	24.5	0.212	83.3	17.3	0.088	92.3	18.8	3
Raji	0.036	83.8	18.4	0.171	65.8	18.6	0.088	87.1	17.8	4
DOHH2	0.115	50.3	19.2	0.874	29.4	19.4	0.399	49.7	20.9	3
SU-DHL-4	0.073	75.5	12.0	ND	23.5	12.5	0.165	76.6	11.8	3
WSU-DLCL2	0.345	65.9	6.3	ND	7.8	8.3	0.577	67.6	7.7	1
Z-138	0.106	41.2	20.6	4.327	28.1	19.5	0.190	38.0	21.2	1
JVM-13	0.146	43.6	13.3	0.769	30.5	13.3	0.458	44.5	13.3	3
REH	0.039	58.3	22.4	0.232	30.6	18.0	0.112	58.1	19.2	3

Jeko-1	0.108	61.6	5.5	0.833	13.2	9.3	0.302	51.5	8.1	2
Wien133	0.015	96.0	8.2	0.199	13.2	7.0	0.013	97.4	7.9	2
697	0.087	57.6	10.9	ND	ND	ND	0.308	65.6	11.1	3
Granta-519	ND	17.4	13.5	ND	15.5	77.8	ND	16.7	13.1	3
RS4;11	0.093	33.9	9.8	ND	14.1	9.9	0.328	29.9	10.1	3
DB	ND	ND	ND	ND	ND	ND	ND	ND	ND	1
NALM-16	0.022	60.9	10.1	0.193	16.2	9.4	0.075	58.6	9.7	3
JVM-3	0.110	42.5	11.6	0.245	19.0	12.8	0.287	40.2	12.2	2
U266	0.052	32.5	9.6	3.889	19.1	10.8	ND	ND	8.7	2
RC-K8	ND	ND	6.6	ND	7.7	ND	ND	8.2	8.6	1
Pfeiffer	ND	ND	ND	ND	ND	ND	ND	ND	ND	2
THP-1	0.075	81.5	12.6	0.051	42.4	6.1	NT	NT	NT	3
Oci-AML3	0.046	90.4	0.0	1.485	26.1	0.0	NT	NT	NT	3
monomac6	0.093	83.9	14.0	0.053	54.1	0.0	NT	NT	NT	3
KG-1	0.104	77.7	0.0	1.401	26.2	2.0	NT	NT	NT	3
ML-2	0.023	99.6	5.1	0.414	95.4	0.0	NT	NT	NT	3
U937	0.057	68.6	0.0	0.140	34.3	0.0	NT	NT	NT	2
Nomo-1	0.039	95.9	6.7	1.937	28.4	1.9	NT	NT	NT	3
MEGAL	0.170	30.1	0.7	ND	ND	ND	NT	NT	NT	3
AML-193	0.032	89.5	0.8	ND	ND	ND	NT	NT	NT	2
MOLM-13	0.017	92.0	0.0	0.290	32.5	9.8	NT	NT	NT	3
HL-60	0.070	52.5	0.0	4.519	10.2	0.0	NT	NT	NT	3
Oci-M1	ND	ND	ND	ND	ND	ND	NT	NT	NT	1
LP-1	0.020	99.4	9.1	0.115	98.2	9.8	NT	NT	NT	3
NCI-H929	0.308	50.9	0.0	0.864	36.1	0.4	NT	NT	NT	3
OPM-2	ND	ND	ND	ND	ND	ND	NT	NT	NT	3
ARH77	ND	ND	ND	ND	ND	ND	NT	NT	NT	3

ND = not determined; NT = not tested

Induction of CDC by wild type and E430G mutated CD38 antibodies using T regulatory cells was also determined. The T regulatory cells were generated as described in Example 8 (Trogocytosis of CD38 from T regulatory cells) and tested in a CDC assay as described above for the tumor cell lines. The percentage of lysis is shown in Figure 17 together with the EC50 values.

Figure 17 demonstrates that IgG1-B induced virtually no lysis of T regulatory cells; while IgG1-B-E430G and IgG1-C-E430G induced lysis of T regulatory cells, where IgG1-C-E430G showed a lower EC50 value compared to IgG1-B-E430G.

CDC in whole blood

Whole blood from a healthy donor was collected in hirudin tubes to prevent coagulation without interference with physiological calcium levels (required for CDC). 50 µL/well was plated into 96-well flat-bottom tissue culture plates (Greiner bio-one). CD38 antibodies and control Abs were serially diluted in RPMI containing 0.2% BSA (0.016-10 µg/mL final antibody concentration in 5x serial dilutions) and 50 µL of diluted Ab was added per well and incubated overnight at 37°C. As a positive control for CDC on B cells, the CD20 Ab IgG1-7D8 was tested with and without 60 µg/mL eculizumab to block CDC. Cells were transferred to polystyrene 96-well round-bottom plates (Greiner bio-one, centrifuged), centrifuged (3 minutes, 1200 rpm) and washed once with 150 µL PBS (B.Braun) per well. Cell pellets were resuspended in 80 µL PBS with 1000x diluted amine reactive viability dye (BD) and incubated 30 minutes at 4°C. Next, cells were washed with 150 µL PBS and incubated with 80 µL PBS containing a cocktail of lymphocyte phenotyping antibodies (1:200 mouse anti-human CD3-EF450 [OKT3, ebioscience], 1:50 mouse anti-human CD19-BV711 [HIB19, Biolegend] and 1:100 mouse anti-human CD56-PE/CF594 [NCAM16.2, BD]) for 30 minutes at 4°C. Cells were washed with 150 µL PBS and incubated 10 minutes at 4°C with 150 µL erythrocyte lysis solution (10 mM KHCO₃ [Sigma], 0.01 mM EDTA [Fluka], 155 mM NH₄Cl [Sigma] dissolved in 1 L of H₂O [B.Braun] and adjusted to pH 7.2). Cells were washed with 150 µL FACS buffer, re-suspended in 100 µL FACS buffer and analyzed on a FACS_Fortessa (BD). The number of viable NK cells (CD56^{pos}, CD3^{neg} and amine reactive viability dye^{neg}), T cells (CD3^{pos} and amine reactive viability dye^{neg}) and B cells (CD19^{pos} and amine reactive viability dye^{neg}) is depicted in **Figure 5**. Data is shown from 1 representative donor out of 5 tested.

Figure 5 demonstrates that CD38 antibodies containing the E430G mutation induce minimal CDC of healthy blood lymphocytes. The positive control CD20 Ab IgG1-7D8 demonstrated specific CDC of CD20-positive B cells, which was completely blocked by the CDC inhibitor eculizumab. Wild type IgG1 CD38 antibodies did not induce CDC of B, T and NK cells. Some CDC was observed for NK cells after incubation with clones B and C containing the E430G mutation (approximately 40% NK cell lysis at the highest concentration with IgG1-B-E430G), but not B and T cells.

Overall, these results indicate that E430G mutated CD38 antibodies have broad CDC activity against a panel of tumor cell lines with variable CD38 expression. CD38 antibodies with an E430G mutation were also tested against lymphocytes obtained from healthy donors, and were shown to only induce up to 40% lysis of NK cells. NK cells express on average 15,000 CD38/cell which is similar to the MM cell line U266. Both cell types are equally sensitive to CDC by E430G mutated CD38 antibodies, indicating that CDC by E430G mutated CD38 antibodies is correlated to CD38 expression. Without being limited to theory, based on these

data, it is believed that the threshold for CDC by E430G-mutated CD38 antibodies lays around 15,000 CD38 molecules/cell. While most B cell tumor cell lines express higher levels of CD38 ranging from 15,000 – 400,000 CD38 molecules /cell, healthy lymphocytes express only 2,000-15,000 CD38 molecules/cell which makes these cells less vulnerable to CDC by E430G mutated CD38 antibodies.

Example 4 – Antibody-dependent cellular cytotoxicity (ADCC) by E430G-mutated CD38 antibodies

The capacity of E430G mutated CD38 antibodies to induce antibody-dependent cellular cytotoxicity (ADCC) was determined by a chromium release assay. Daudi cells were collected (5×10⁶ cells/mL) in 2 mL culture medium (RPMI 1640 supplemented with 0.2% BSA), to which 100 µCi ⁵¹Cr (Chromium-51; PerkinElmer) was added. Cells were incubated in a water bath at 37°C for 1 hour while shaking. After washing of the cells (twice in PBS, 1500 rpm, 5 min), the cells were resuspended in culture medium and counted by trypan blue exclusion. Cells were diluted to a density of 1×10⁵ cells/mL and pipetted into 96-well round-bottom microtiter plates (Greiner Bio-One), and 50 µL of a concentration series of (0.005-10 µg/mL final concentrations in 3-fold dilutions) CD38 or isotype control antibody, diluted in culture medium was added. Cells were pre-incubated with Ab at room temperature (RT) for 15 min.

Meantime, peripheral blood mononuclear cells (PBMCs) from healthy volunteers (Sanquin) were isolated from 45 mL of freshly drawn heparin blood (buffy coats) using lymphocyte separation medium (Bio Whittaker) according to the manufacturer's instructions. After resuspension of cells in culture medium, cells were counted by trypan blue exclusion and diluted to a density of 1×10⁷ cells/mL.

After the pre-incubation of target cells with Ab, 50 µL effector cells was added, resulting in an effector to target cell ratio of 100:1. Cells were incubated for 4 hours at 37°C and 5% CO₂. For determination of maximal lysis, 50 µL ⁵¹Cr-labeled Daudi cells (5,000 cells) were incubated with 100 µL 5% Triton-X100; for determination of spontaneous lysis (background lysis), 5,000 ⁵¹Cr-labeled Daudi cells were incubated in 150 µL medium without any antibody or effector cells. The level of antibody-independent cell lysis was determined by incubating 5,000 Daudi cells with 500,000 PBMCs without antibody. Plates were centrifuged (1200 rpm, 10 min) and 75 µL of supernatant was transferred to micronic tubes, after which the released ⁵¹Cr was counted using a gamma counter. The percentage of antibody-mediated lysis was calculated as follows:

% specific lysis = (cpm sample - cpm spontaneous lysis)/(cpm maximal lysis - cpm spontaneous lysis) wherein cpm is counts per minute.

Figure 6 shows that all CD38 Abs were able to induce lysis of Daudi, as indicated by the increased lysis that was seen for CD38 Abs in comparison to the isotype control (IgG1-b12-E430G). Already at the lowest antibody concentration cell lysis was noted, suggesting that antibodies should have been further diluted in order to observe a dose-dependent effect.

- 5 CD38 antibodies that contain an E430G mutation showed lower maximum lysis compared to wild type antibodies.

The above chromium release assay was repeated with peripheral blood mononuclear cells from different healthy volunteers (effector cells), the following target cells: Daudi, Wien-133, Granta 519 and MEC-2, and with the antibodies IgG1-B-E430G, IgG1-B, IgG1-C-E430G,

- 10 IgG1-C and IgG1-b12-E430G. The results are shown in Figure 18.

Figure 18 shows that all CD38 Abs were able to induce lysis of Daudi, Wien-133, Granta 519 and MEC-2 cells as indicated by the increased lysis that was seen for CD38 Abs in comparison to the isotype control (IgG1-b12-E430G). In most instances dose-dependent target cell lysis was seen, but some variation was observed between different PBMC donors.

- 15 The ability of CD38 antibodies to induce ADCC was further evaluated using a luminescent ADCC reporter bioassay (Promega, Cat # G7018) that detects FcγRIIIa (CD16) crosslinking, as a surrogate for ADCC. As effector cells, the kit provides Jurkat human T cells that are engineered to stably express high affinity FcγRIIIa (V158) and a nuclear factor of activated T cells (NFAT)-response element driving expression of firefly luciferase. Briefly, Daudi or T
- 20 regulatory cells (5,000 cells/well) were seeded in 384-well white Optiplates (Perkin Elmer) in ADCC Assay Buffer [RPMI-1640 medium [(Lonza, Cat # BE12-115F) supplemented with 3.5% Low IgG Serum] and incubated for 6 hours at 37°C/5%CO₂ in a total volume of 30 μL containing antibody concentration series (0.5-250 ng/mL final concentrations in 3.5-fold dilutions) and thawed ADCC Bioassay Effector Cells. After adjusting the plates for 15 minutes
- 25 to room temperature (RT), 30 μL Bio Glo Assay Luciferase Reagent was added and plates were incubated for 5 minutes at RT. Luciferase production was quantified by luminescence readout on an EnVision Multilabel Reader (Perkin Elmer). Background levels were determined from wells to which only target cells and antibody (no effector cells) was added. As negative control, wells containing only target and effector cells (no antibody) were used.

- 30 **Figure 7** shows the results obtained with the Daudi cells, which show that CD38 antibodies were highly effective in inducing dose-dependent FcγRIIIa cross-linking as determined in the reporter assay. CD38 antibodies that contained an E430G mutation showed lower maximum cross-linking compared to the respective wild type antibodies, which was in line with results obtained for the chromium release assay.

Figure 19 shows the results obtained with the T regulatory cells, which show that CD38 antibodies were highly effective in inducing dose-dependent FcγRIIIa cross-linking as determined in the reporter assay. CD38 antibodies that contained an E430G mutation showed lower maximum cross-linking compared to the respective wild type antibodies.

5 *Example 5 – Antibody-dependent cellular phagocytosis (ADCP) by E430G-mutated CD38 antibodies*

The capacity of E430G mutated CD38 antibodies to induce antibody-dependent cellular phagocytosis was adapted from Overdijk M.B. et al. mAbs 7:2,311-320. Macrophages were obtained by isolating PBMCs from healthy volunteers (Sanquin) using lymphocyte separation
10 medium (Bio Whittaker) according to manufacturer's instructions. From the PBMCs, monocytes were isolated via negative selection, using Dynabeads Untouched Human Monocyte isolation kit (Invitrogen). The isolated monocytes were cultured 3 days in serum-free dendritic cell medium (CellGenix GmbH) supplemented with 50 ng/mL GM-CSF (Invitrogen), followed by 2 days in serum-free dendritic cell medium supplemented with 100
15 ng/mL GM-CSF, to induce macrophage differentiation. The differentiated macrophages were detached using versene (Life Technologies) and cell scraping and characterized by flow cytometry for staining with CD1a-FITC (BD), CD14-PE/Cy7 (BD), CD40-APC/H7 (BD), CD80-APC (Miltenyi biotec), CD83-PE (BD) and CD86-PerCP-Cy5.5 (Biolegend). Macrophages were seeded at 100,000 cells per well into 96-well flat-bottom culture plates (Greiner bio-one) and
20 allowed to adhere overnight at 37°C in serum-free dendritic cell medium supplemented with 100 ng/mL GM-CSF.

Target cells (Daudi) were labeled with PKH-26 (Sigma) according to manufacturer's instructions, opsonized with 10 µg/mL CD38 antibody (30 minutes at 4°C), washed three times with FACS buffer and added to the macrophages at an effector:target (E:T) ratio of
25 5:1. The plate was briefly spun at 300 rpm to bring the effector cells and target cells in close proximity and incubated 45 minutes at 37°C. Next, macrophages were collected using versene and stained with CD14-BV605 (biolegend) and CD19-BV711 (biolegend). Phagocytosis was depicted as the percentage of CD14-positive macrophages that were also positive for PKH-26, but negative for CD19 (to exclude macrophages that are only attached
30 to Daudi cells), measured on a flow cytometer (BD).

Figure 8 shows that all CD38 Abs were able to induce ADCP of Daudi cells, as indicated by the increased percentage of PKH-29^{pos}, CD14^{pos} and CD19^{neg} macrophages that was seen for CD38 Abs in comparison to the isotype controls (IgG1-b12 and IgG1-b12-E430G). Depending on the donor used, CD38 antibodies that contain an E430G mutation showed a higher
35 percentage of PKH-29^{pos}, CD14^{pos} and CD19^{neg} macrophages compared to wild type

antibodies, indicating CD38-Ab mediated phagocytosis can be increased by introducing the E430G mutation.

Example 6 – Induction of apoptosis by CD38 antibodies on tumor cell lines

- Apoptosis induction by CD38 antibodies was investigated by overnight incubation of tumor cell lines with CD38 antibody followed by live/dead analysis on a flow cytometer. Cells, resuspended in RPMI containing 0.2% BSA, were seeded at 100,000 cells/well in 96 well flat-bottom tissue culture plates (Greiner bio-one). Serial dilutions (0.01-10 µg/mL final antibody concentration in 4x serial dilutions) of CD38 or control antibodies were added in the absence or presence of 10 µg/mL goat-anti-human IgG1 (Jackson) to provide additional Fc-cross-linking. Cells were incubated overnight at 37°C, washed/centrifuged twice using FACS buffer (PBS/0.1% BSA/0.01% Na-Azide), and resuspended in FACS buffer supplemented with 1:4000 diluted Topro-3-iodine (Life Technologies). Cell viability was analyzed on a FACS_Fortessa (BD) and depicted as the percentage of apoptotic (topro-3-iodine positive) cells.
- Figure 9** shows that wild type and E430G mutated CD38 antibodies did not induce apoptosis alone, but the addition of an Fc-cross-linking antibody resulted in approximately 30% of apoptosis. No difference was seen between wild type and E430G mutated CD38 antibodies.

Example 7 – Inhibition of CD38 enzyme activity in the absence of PBMCs

Inhibition of CD38 cyclase activity

- CD38 is an ecto-enzyme that converts NAD into cADPR and ADPR. These activities are dependent on the presence of H₂O. When H₂O is present, NAD is converted into ADPR, (glycohydrolase activity) and cADPR is converted into ADPR (hydrolase activity). About 95% of NAD is converted into ADPR through (glyco)hydrolase activity. In the absence of H₂O, CD38 turns NAD into cADPR using its cyclase activity. To measure inhibition of CD38 enzyme activity, NAD derivatives were used that become fluorescent after being processed by CD38.

Figure 10 illustrates the enzyme activities of CD38.

- First, inhibition of CD38 cyclase activity was measured using nicotinamide guanine dinucleotide sodium salt phosphodiesterase (NGD, Sigma) as a substrate for CD38. As a source of CD38, tumor cell lines with different CD38 expression levels were used as well as recombinant his-tagged extracellular domain of CD38 (hisCD38). Tumor cells (Daudi and Wien133) were harvested and washed with 20 mM Tris-HCL. Cells were resuspended in 20

mM Tris-HCL and 200,000 cells/well were seeded in 96-well white opaque plates (PerkinElmer) in 100 μ L/well. HisCD38 was seeded at 0.6 μ g/mL in 100 μ L/well 20 mM Tris-HCL. CD38 antibodies were diluted to 100 μ g/mL in 20 mM Tris-HCL and 10 μ L was added to the cells and hisCD38 (final concentration is 9 μ g/mL) and incubated for 20 minutes at room temperature. Control wells were incubated with b12 antibody instead of CD38 antibody, or with no antibody at all. Next, 10 μ L (80 μ M) NGD diluted in 20 mM Tris-HCL was added to the plate and fluorescence was immediately measured on the Envision multilabel reader (PerkinElmer) using excitation 340nm and emission 430nm. The conversion of NGD was followed real time, by measuring fluorescence at the indicated time points in **Figure 11** until a plateau is reached. For hisCD38, fluorescence was measured every 3 minutes for 27 minutes, for Daudi cells fluorescence was measured after 5, 15, 30, 60, 120 and 185 minutes and for Wien133, fluorescence was measured after 5, 15, 30, 60, 150, 220, 300 and 360 minutes. Inhibition of CD38 cyclase activity was depicted as percent inhibition compared to control, where control is a sample with hisCD38 and NGD, but no Ab. One representative experiment is depicted for each condition tested.

Figure 11A demonstrates that NGD was rapidly converted through hisCD38 cyclase activity. The conversion was complete after approximately 9 minutes. In the presence of CD38 Ab B the maximum percent of NGD conversion was reduced with ~25%, in the presence of CD38 Ab C the maximum percent of NGD conversion was reduced with ~50%, while CD38 Ab A had no effect on the total turnover of NGD. The inhibition of CD38 cyclase activity was not affected by presence of the E430G mutation. Similar results were seen in **Figures 11B** and **11C**, where NGD conversion by CD38 present on Daudi and Wien133 cells were measured. The kinetics of NGD conversion were a bit slower on Daudi and especially Wien133 cells, which is likely correlated to less CD38 molecules being present. Nevertheless, 25% inhibition of CD38 cyclase activity was induced by Ab B (~25% inhibition) and ~40% inhibition of CD38 cyclase activity was induced by Ab C, while Ab A showed no effect. Wild type antibodies and E430G mutated antibodies showed the similar results, indicating that the E430G mutation does not impact antibody-mediated inhibition of CD38 cyclase activity.

Example 8 – Antibody-dependent trogocytosis by E430G mutated CD38 antibodies

Trogocytosis by E430G mutated CD38 antibodies on Daudi cells

The capacity of E430G mutated CD38 antibodies to induce trogocytosis on Daudi cells was evaluated. Macrophages were obtained by isolating PBMCs from healthy volunteers (Sanquin) using lymphocyte separation medium (Bio Whittaker) according to manufacturer's instructions. From the PBMCs, monocytes were isolated via negative selection, using Dynabeads Untouched Human Monocyte isolation kit (Invitrogen). The isolated monocytes

- were cultured 3 days in serum-free dendritic cell medium (CellGenix GmbH) supplemented with 50 ng/mL GM-CSF (Invitrogen), followed by 2 days in serum-free dendritic cell medium supplemented with 100 ng/mL GM-CSF, to induce macrophage differentiation. The differentiated macrophages were detached using versene (Life Technologies) and cell
- 5 scraping and characterized by flow cytometry for staining with CD1a-FITC (BD), CD14-PE/Cy7 (BD), CD40-APC/H7 (BD), CD80-APC (Miltenyi biotec), CD83-PE (BD) and CD86-PerCP-Cy5.5 (Biolegend). Macrophages were seeded at 100,000 cells per well into 96-well flat-bottom culture plates (Greiner bio-one) and allowed to adhere overnight at 37°C in serum-free dendritic cell medium supplemented with 100 ng/mL GM-CSF.
- 10 Target cells (Daudi) were labeled with PKH-26 (Sigma) according to manufacturer's instructions, opsonized with 10 µg/mL CD38 antibody (30 minutes at 4°C), washed three times with FACS buffer and added to the macrophages at an effector:target (E:T) ratio of 5:1. The plate was briefly spun at 300 rpm to bring the effector cells and target cells in close proximity and incubated 45 minutes at 37°C.
- 15 **Figure 21** illustrates the assay set-up used to measure trogocytosis.

CD38 expression and human IgG staining were determined on Daudi cells by incubation with FITC-conjugated CD38 clone A and goat anti-human IgG-FITC (Southern Biotech) respectively. CD38 clone A was used to stain CD38 because this Ab recognizes a non-overlapping epitope on CD38 compared to clones B and C.

- 20 **Figure 12** shows that CD38 expression on Daudi cells was significantly reduced after 45 minute co-culture with macrophages and CD38 antibodies. The reduction in CD38 expression was strongest with E430G mutated CD38 antibodies. The same trend was seen for human IgG staining on antibody opsonized Daudi cells.

25

Trogocytosis by E430G mutated CD38 antibodies on T regulatory cells

- T regulatory cells (Tregs) with high CD38 expression are more immune suppressive compared to Tregs with intermediate CD38 expression (Krejci J. et al. Blood 2016 128:384-394). Therefore strategies to reduce CD38 expression on Tregs might reduce the immune
- 30 suppressive effects of these cells. We investigated if E430G mutated CD38 antibodies can reduce CD38 expression on Tregs through trogocytosis. Tregs were isolated from PBMCs from

healthy volunteers (Sanquin) using lymphocyte separation medium (Bio Whittaker) according to manufacturer's instructions. From the PBMCs, CD4⁺ T cells were isolated via negative selection, followed by enrichment for CD4⁺ CD25⁺ T regulatory cells, using Treg isolation kit (Miltenyi) according to manufacturer's instructions. Subsequently, Tregs were expanded at

5 5x10⁴ cells/mL in serum-free dendritic cell medium supplemented with 5% human serum (Sigma), 1000 U/mL IL-2 (peprotech), 100 ng/mL rapamycin (Sigma) and CD3/CD28 coated beads (Gibco) at a bead:cell ratio of 4:1 for 20 days at 37°C. Every 3 to 4 days the cell density was adjusted to 5x10⁵ cells/mL using serum-free dendritic cell medium supplemented with 1000 U/mL IL-2 and 100 ng/mL rapamycin. T regulatory phenotype was followed over

10 time using flow cytometry staining with the following antibodies: CCR7-BV785 (Biolegend), CD62L-FITC (BD), CD4-APC/efluor780 (e-biosciences), CD25-PerCP/Cy5 (Biolegend), Foxp3-PE/CF594 (BD), CTLA4-efluor660 (e-biosciences), CD127-PE/CY7 and CD38-GV605 (Biolegend).

To evaluate Ab induced trogocytosis of CD38 from Tregs, Tregs (target cells) were co-

15 cultured with PBMCs (effector cells) and CD38 expression was monitored on the Tregs. In brief: PBMCs were isolated from buffy coats (Sanquin) using lymphocyte separation medium (Bio Whittaker) according to manufacturer's instructions and seeded in RPMI-1640 medium (Lonza) supplemented with 0.2% BSA at a density of 5x10⁵ cells per well and cultured 3 days to allow monocytes to adhere. Tregs were labeled with 0.25 µM CellTrace far red (CTFR)

20 according to manufacturer's instruction and pre-incubated with E430G mutated CD38 Ab for 10 minutes at 37°C. Tregs were washed and 1x10⁵ Ab-opsonized cells per well were transferred to the plate with PBMCs. The PBMCs and Tregs were briefly spun at 300 rpm to bring the cells in close proximity and incubated for 23 hours at 37°C. Trogocytosis of CD38 was measured by analyzing CD38 expression with FITC-conjugated CD38 clone A on CTFR-

25 positive Tregs with flow cytometry.

Figure 13 shows that CD38 expression on T regulatory cells was reduced after incubation with E430G mutated CD38 antibodies and PBMCs. Without PBMCs, no reduction of CD38 expression on T regulatory cells was seen, strongly suggesting trogocytosis. Furthermore, in presence of PBMCs, IgG1-B did not induce trogocytosis of CD38, while a strong reduction in

30 CD38 expression was induced by E430G mutated B and C. This suggests that E430G mutated CD38 antibodies induce enhanced trogocytosis of CD38.

Example 9 - Anti-tumor activity of a E430G mutated CD38 antibody C in patient derived Diffuse Large B Cell Lymphoma models

Patient derived Diffuse Large B Cell Lymphoma (DLBCL) cells were inoculated subcutaneous

35 in CB17.SCID mice and antibody treatment (2 weekly doses of 5 mg/kg IgG1-C-E430G,

- injected intravenously; PBS was used as negative control) was initiated when tumors reached a mean volume of approximately 150-250 mm³. Tumor volumes were measured in two dimensions using a caliper, and the volume was expressed in mm³ using the formula: $V = (L \times W \times W)/2$, where V is tumor volume, L is tumor length (the longest tumor dimension) and W is tumor width (the longest tumor dimension perpendicular to L), and depicted over time in Figure 20. Each treatment group consists of a single mouse. To calculate a response value the following formula was used; (tumor volume of IgG1-C-E430G treated mouse on day X - tumor volume of IgG1-C-E430G treated mouse on day 0) / (tumor volume of control mouse on day X - tumor volume of control mouse on day 0).
- X = the latest day in the period between day 7 to day 25 on which both animals were alive and tumor measurement was performed.

The response values are depicted in Table 5 as well as CD38 mRNA expression. The models that had the highest CD38 mRNA levels also showed the best response. This could also be seen from the graphs in Figure 20. Thus two weekly doses of IgG1-C-E430G reduced the tumor growth in two out of five tested DLBCL PDX models that had highest CD38 mRNA expression.

Table 5 Overview of CD38 mRNA expression and calculated response value for five DLBCL PDX models. A low response value indicates tumor regression.

Model	CD38 (determined by RNASeq: log ₂ (TPM value + 1))	Response (ΔT/ΔC)	Response calculated for day;
Ly12638	6,427	-11%	15
Ly11212	6,066	-2%	11
Ly13976	6,017	54%	13
Ly13693	4,796	58%	22
Ly14862	0	83%	11

Example 10 - IgG1-C-E430G induces potent complement-mediated cytotoxicity in bone marrow mononuclear cells from newly diagnosed MM patients

- Bone marrow mononuclear cells (BM-MNC) were isolated by Ficoll-Hypaque density-gradient from full bone marrow aspirates from 3 newly diagnosed MM patients and 1 relapsed/refractory MM patient and frozen at -80°C until use. On the day of use, BM-MNC were thawed, viable cells were counted and plated in 96-well plates. Cells were incubated with serial dilutions (0.01 – 10 µg/mL) of IgG1-C-E430G or Darzalex® for 15 min at room temperature on a plate shaker. As negative controls, cells were untreated or were incubated

with 10 µg/mL IgG1-b12. As a source of complement, 20% normal human serum was added 45 min prior to FACS measurements, in which absolute numbers of cells were determined using flow cytometric count beads as a constant. To determine the overall percentages lysis, the untreated control wells were used as control values. The percentage multiple myeloma cell lysis was determined relative to controls using the following equation:

$$\% \text{ cell lysis} = (1 - (\text{number of surviving cells in antibody-treated samples} / \text{number of surviving cells in untreated controls})) \times 100\%$$

Figure 22A and B show that IgG1-C-E430G induced higher levels of lysis in two BM-MNC samples from newly diagnosed MM patients compared to Darzalex®. The maximal lysis induced by IgG1-C-E430G was in the range of 84-90% compared to a maximal lysis in the range of 31-55% induced by Darzalex®. In two other BM-MNC samples, one from a relapsed/refractory MM patient that did not receive Darzalex® as part of prior therapy (Figure 22C) and one from a newly diagnosed MM patient (Figure 22D), no induction of CDC was noted with IgG-C-E430G or Darzalex® (Figure 22C and D).

Example 11 - Antibody formulation

Studies were performed to evaluate the effect of buffer identity, solution pH, and excipients on the protein stability of IgG1-C-E430G. IgG1-C-E430G with a VH comprising SEQ ID NO:1, a VL comprising SEQ ID NO:5, a CH comprising SEQ ID NO:46 and a CL comprising SEQ ID NO:37 was expressed in CHO cells.

Methods

Biophysical Study:

A biophysical study evaluated IgG1-C-E430G (2 mg/mL) in different buffer/excipient/pH combinations. Two buffer types (acetate and histidine), between pH 5.5 – 6.5, with four types of excipients (NaCl, Arginine, Sucrose, Sorbitol) were prepared for the study (Table 5).

The thermal and conformational stability of IgG1-C-E430G was assessed by differential scanning fluorimetry (DSF) and the aggregate profile and particle sizing was determined by dynamic light scattering (DLS).

Table 5. Biophysical Study (BS) Formulations

BS ID	Formulation	Excipient	pH
F1	20 mM Acetate	-	5.5
F2		NaCl	5.5
F3		Arginine	5.5
F4		Sorbitol	5.5
F5		Sucrose	5.5
F6	20 mM Histidine	-	5.5
F7		NaCl	5.5
F8		Arginine	5.5
F9		Sorbitol	5.5
F10		Sucrose	5.5
F11		-	6.0
F12		NaCl	6.0
F13		Arginine	6.0
F14		Sorbitol	6.0
F15		Sucrose	6.0
F16		-	6.5

Physical Stress and Surfactant Study:

- Following the biophysical study, 6 select formulations were evaluated to determine the protection provided during freeze-thaw cycles and agitation of IgG1-C-E430G. The effect of a surfactant in the formulation was also evaluated by spiking in Polysorbate 80 (PS80) into each of the 6 formulations, resulting in a total of 12 formulations for evaluation (Table 6).

- IgG1-C-E430G was buffer exchanged into 6 formulations by centrifugation filtration and subjected to either five freeze-thaw cycles or 48 hours of agitation on a table top plate shaker at 400 RPM. A third set of samples, held on the benchtop for 48 hours at RT, were used as controls. For surfactant formulations, the PS80 was spiked-in after the buffer exchange.

Samples were analyzed using the following analytical techniques: appearance, size exclusion chromatography (SEC), A_{280} , DLS and A_{550} .

Table 6. Physical Stress Study (PSS) Formulations

PSS ID	Formulation	Excipient	pH	% PS80
F2	20 mM Acetate	NaCl	5.5	-
F5		Sucrose	5.5	-
F11	20 mM Histidine	-	6.0	-
F12		NaCl	6.0	-
F14		Sorbitol	6.0	-
F15		Sucrose	6.0	-
F2	20 mM Acetate	NaCl	5.5	0.04%
F5		Sucrose	5.5	0.04%
F11	20 mM Histidine	-	6.0	0.04%
F12		NaCl	6.0	0.04%
F14		Sorbitol	6.0	0.04%
F15		Sucrose	6.0	0.04%

Stability Study:

The formulation shown in Table 7 was selected for evaluation of IgG1-C-E430G at 20 mg/mL under nominal (2-8°C) and accelerated (25°C/60% relative humidity (RH) and 40°C/75%RH) conditions up to 12 weeks. To confirm product quality of the optimal formulation following physical stress, at the initial condition, one additional sample underwent five freeze-thaw cycles and one sample underwent agitation (48 hours at 400 RPM).

The following analytical techniques were used: appearance, pH determination; A_{280} (single replicate), SEC, imaging capillary isoelectric focusing (icIEF), and reduced and non-reduced capillary electrophoresis.

Table 7. Stability Study Formulation

Antibody conc. (mg/mL)	Formulation	Excipient	pH	Surfactant
20	20 mM Histidine	250 mM sorbitol	6.0	0.04% PS80

Analytical Methods

Differential Scanning Fluorimetry (DSF):

DSF analysis utilizes increasing thermal stress to induce protein unfolding to assess thermal and colloidal stability. The unfolded state transitions caused by increased thermal stress are detected by changes in intrinsic fluorescence of the protein due exposure of buried hydrophobic residues upon changes in environment conditions. As tryptophan residues are exposed, the maximum emission wavelength moves to longer wavelengths. Barycentric mean (BCM), the wavelength at which the fluorescence emission spectrum is equally divided, is plotted, showing the conformational change of the protein over temperature. DSF analysis provides the onset of unfolding temperature (T_{onset}) and the melting temperature (T_m) values, which are generated from the BCM curves. The T_{onset} provides the calculated temperature at which the protein begins to unfold. The T_m value is a calculated temperature-dependent transition midpoint of the protein from the folded state to the unfolded state.

During DSF analysis, static light scattering (SLS) measurements are determined at 266 nm and 473 nm to assess protein colloidal stability. The intensity of static light scattering from the lasers used to illuminate the sample is proportional to the presence of particles on the same order of magnitude as the incident light. This analysis is therefore sensitive to protein aggregation over the temperature ramp. The static light scattering is measured at 266 nm, to detect smaller aggregate particle sizes, as well as 473 nm, for the detection of larger aggregate species. The onset of aggregation temperature (T_{agg}) is determined from these data, which is the temperature at which the protein begins to aggregate. These data are best analyzed by large changes in count intensity – higher counts indicate more light has been scattered due to the association of protein aggregates. Over an increasing temperature ramp, changes in SLS count data in the 10^3 scale is typically attributed to significant protein aggregation, minimal changes in SLS counts are attributed to partial aggregation, and no change in SLS counts over the temperature ramp is indicative of negligible protein aggregation.

Dynamic Light Scattering (DLS):

DLS analysis assesses protein size and aggregation at room temperature. For DLS analysis, time autocorrelation functions of scattered light are determined, and a single particle size is assumed with a single exponential cumulant fitting of data. The cumulant fits are used to determine the size distribution of particles in solution. The reportable values are polydispersity and hydrodynamic radius (diameter). For protein samples comprised of a single distribution of monomer, the sample is considered monodisperse, however, for

samples containing multiple particle size populations, the samples are considered polydisperse. The polydispersity index is a measure of the width of the particle size distribution - the higher the polydispersity index, the wider the distribution of particles. Therefore, samples with high PDI are typically found to contain higher order polymers or large aggregates. The hydrodynamic radius of a non-spherical protein particle is the diameter of a sphere that has the same translational diffusion speed as the particle. The diffusion coefficient depends on the molecular weight of the particle, the surface structure, as well as the concentration and type of ions in the formulation. A larger hydrodynamic radius in a monodisperse size distribution can be attributed to the presence of higher order oligomers (e.g. tetramers) in solution, but not large aggregates.

For the present analyses, DSF and DLS data for each sample were collected in the same run on an Uncle instrument (Unchained Labs). Briefly, samples of 2 mg/mL antibody solution (diluted in the respective formulation buffer) were analyzed, typically at 48 samples per run. DLS data was collected at the lowest temperature, typically 25°C. Then, for DFS data, a thermal ramp is started, typically at 1°C/min, and fluorescence measured continually for each sample throughout the temperature ramp.

Visual Appearance:

Antibody samples were equilibrated to room temperature. Glass vials were inspected to ensure they were clean and free of scratches or foreign matter. If applicable, 1 mL of the sample was transferred into an appropriate glass vial. 1 mL of water in a similar glass vial was used for comparison. Appearance was evaluated against a clean white and black background with ambient lab lighting. Color was evaluated against a white background in comparison to water. Clarity was evaluated in comparison to water against a white and black background. Particulates were evaluated by gently inverting the vial, ensuring air bubbles were not introduced, then observing for ~ 5 seconds in front of a white and black background.

pH:

Samples were equilibrated to room temperature. Typically, 100-250 µL of sample was transferred into a tube for pH measurement at 23-27 °C. The pH probe was immersed in the sample and the pH is measured. pH probes were calibrated daily.

Protein Concentration by UV A₂₈₀:

Protein concentration was determined using an UV/Vis Spectrophotometer (Agilent or equivalent system) by measuring the light absorbance of the sample at 280 nm (A_{280}) using 320 nm (A_{320}) as the reference wavelength. Samples were diluted to approximately 0.6 mg/mL using dilution buffer (12.6 mM sodium phosphate monobasic, 140 mM sodium chloride, pH 7.3) and transferred to UVette disposable cuvettes (Eppendorf). The spectrophotometer was blanked using formulation buffer, and then used to measure the absorbance for all wavelengths over the range of 240 to 400nm for the blank and prepared samples. The results for A_{280} and A_{320} were used to determine the total protein concentration using the Beer-Lambert Law.

10 Turbidity by A_{550}

Turbidity was determined using an UV/Vis spectrophotometer (Agilent 8453 or 8454 or equivalent system) by measuring the light absorbance of the sample at 550 nm with no background correction in a 1cm cuvette (UVette disposable cuvette; Eppendorf) with undiluted material.

15 Size Exclusion Chromatography (SEC)

SEC is used to evaluate initial purity of the drug product as well as the degree of fragmentation, observed as low molecular weight (LMW) species, and aggregation, observed as high molecular weight (HMW) species. Relative peak areas for the chromatogram produced by the DAD are used to determine the purity of the monomer relative to (HMW) species which elute before the monomer and (LMW) species which elute after the monomer. The results reported for this method are the % monomer purity, the total of all peak eluted prior to the monomer as % total HMW species, the total of all peaks eluting after the monomer reported as % total LMW species, and the total of all non-monomer species reported as % total impurities.

Purity evaluation by SEC was performed using a high-performance liquid chromatography (HPLC) system (Agilent 1100/1200 series or equivalent) with 100 mM sodium phosphate, 100 mM sodium sulfate, pH 6.8 mobile phase and a TOSOH TSK-gel G3000SWxl (7.8 x 300 mm) column stationary phase (Sigma). Sample preparation consisted of dilution in mobile phase to 1.0 mg/mL. Instrument parameters consisted of a 50 μ L (50 μ g load) sample injection volume, a flow rate of 1.0 mL/ min, column temperature of 27.5°C, an autosampler temperature of 5 $\pm$ 3°C, and a sample run time of 20 minutes. Data was collected at a wavelength of 280 nm with bandwidth of 8 nm using a diode array detector (DAD).

Imaged Capillary Isoelectric Focusing (icIEF)

Imaged capillary isoelectric focusing was used to determine charge heterogeneity and the isoelectric point of antibody samples. icIEF detects focused protein zones using a whole capillary UV detector. During an icIEF separation, a voltage is applied to a coated capillary filled with sample, pI (isoelectric point) markers, methyl cellulose, and carrier ampholytes
 5 allowing for separation of the protein species according to its pI. The separation capillary is connected to an inlet and outlet capillary using hollow fiber membranes located in the anode (acidic) and cathode (basic) tanks. This allows for electrical communication between the tanks and the inner volume of the separation capillary. High voltage applied across the tanks allows for charge-based separation of the protein species. This method is used to observe
 10 changes in the charge heterogeneity of the formulated antibody due to chemical changes such as deamidation over the course of the stability study.

The resulting electropherogram (absorbance vs pH) captured at the end of the separation is used to generate charge heterogeneity results based on the percentage of relative area for each peak compared to the total area. The results reported for this method include the %
 15 main peak, the %total acidic variants (variants with pI lower than the main peak), and the % total basic variants (variants with pI higher than the main peak). Additionally, two protein markers with known pI values are used to determine the pI for each peak, and the pI of the main peak is reported.

The analysis utilized a cIEF cartridge and Maurice cIEF Chemical Test Kit from ProteinSimple.
 20 Samples and reference standard were diluted to 3 mg/mL in purified water (MilliQ or equivalent). Master mix was prepared following the component list shown in the following, with the formula scaled to prepare a volume sufficient for all samples:

Component	Volume (μL)	Final Composition
1% methyl cellulose	35	0.35% (v/v)
8 M urea	40	3 M
Pharmalyte 8-10.5 (GE Healthcare)	6	6.0% (v/v)
Pharmalyte 3-10 (GE Healthcare)	2	2.0% (v/v)
6.14 pI marker (ProteinSimple)	0.5	0.01% (v/v)
10.10 pI marker (ProteinSimple)	0.5	0.01% (v/v)
MilliQ Water	6	N/A
Total	90	

The sample was then diluted to its final concentration (0.3 mg/mL) by combining 10 μL of
 25 sample with 90 μL of master mix. A blank was also prepared using 10 μL of water in place of sample. The Maurice cIEF system suitability standard from the test kit was prepared per manufacturer instructions. All preparations were then transferred to a Maurice 96 well plate in the following order: system suitability, blank, reference standard, sample preparations.

The buffer vial preparation consisted of preparing ProteinSimple pressure capped 2mL vials containing 0.5 mL of fluorescence calibration standard, 2 mL of MilliQ water, 2 mL of 0.5% methyl cellulose, and an empty vial as well as a vial with 2mL MilliQ water and a clear screw cap. These vials were then loaded into the instrument per the description in the software.

- 5 The cartridge was prepared by loading 2 mL of catholyte into the red port and 2mL of anolyte into the white port.

Capillary electrophoresis (CE)

- 10 In CE, protein species are separated by migration through gel under the influence of an electric field. The gel contains a cross-linked polymer network which acts a molecular sieve, with smaller molecules moving more quickly through the gel.

SDS is used to unfold the proteins and to apply a uniform charge to allow migration in electric field. The reducing agent (DTT) is added to disrupt disulfide bonds. The Protein Express Dye binds to the protein and fluoresces when exposed to intense light, which is detected by the instrument as the fragments pass through the sensor.

- 15 *Reduced Microchip Capillary Electrophoresis (R CE-SDS)*

Results are determined by comparing the relative areas for each peak of interest to the total area of all peaks. Reported results are the % light chain (% LC), % heavy chain (%HC), % non-glycosylated heavy chain (% NGHC), % purity (% LC + % HC), and the % total related substances/ impurities (sum of all peaks excluding % LC and % HC).

- 20 The analysis utilized the Caliper Lab Chip CXII system, the Protein Express Assay Lab Chip, and the Caliper Protein Express Reagent kit, all from Perkin Elmer, to perform purity evaluation by Lab on a Chip (LoC) Capillary electrophoresis sodium dodecyl sulfate (CE-SDS), using Dithiothreitol (DTT) as the reducing agent. Samples and reference standard were diluted to 1 mg/mL in phosphate buffered saline (PBS) pH 7.4. A reducing working solution
- 25 was prepared by diluting 24.5 μ L 1M DTT in 700 μ L of HT Protein Express Sample Buffer to a final concentration of 33.8 mM. Samples were reduced by combining 5 μ L of diluted sample with 7 μ L of the reducing working solution in a microcentrifuge tube, followed by centrifugation at 3000 x g for 1 minute and heating at $70 \pm 2^\circ\text{C}$ for 3 minutes on a heat block. The samples were then cooled to room temperature followed by centrifugation at
- 30 3000 x g for 1 minute. The sample preparations were then diluted with 32 μ L of MilliQ water and transferred to a 96 well plate. A blank was prepared using 5 μ L of PBS pH 7.4 and the same sample preparation procedure to ensure there were no interfering peaks.

Chip preparation was performed with reagents added to the lab chip. Gel-Dye mix was prepared by adding 18 μL of HT Protein Express Dye Solution to 520 μL of HT Protein Express Gel Matrix followed by centrifugation in the provided spin filters at 9300 x g for 5 minutes. Destain solution was prepared by adding 250 μL of HT Protein Express Gel Matrix into a spin filter and centrifuging at 9300 x g for 5 minutes. HT Express Ladder was prepared by heating 12 μL at $100 \pm 2^\circ\text{C}$ for 5 minutes on a heat block, followed by cooling and the addition 120 μL of MilliQ water. The ladder was transferred to a 0.2 mL PCR tube and loaded into the designated position in the instrument. Chip preparation is sufficient to run a 96 well plate. The chip and sample plate were loaded into the instrument and samples are run.

10 *Non-Reduced Microchip Capillary Electrophoresis (NR CE-SDS)*

Results are determined by comparing the relative areas for each peak of interest to the total area of all peaks. Reported results are the % purity of the main peak, % front side related substance/ impurities (peaks with molecular weights lower than the main peak), % back side related substances/ impurities (peaks with molecular weights higher than the main peak), and the % total related substance/ impurities (%TRS/ I).

The analysis utilized the Perkin Elmer Caliper Lab Chip CXII system, the Protein Express Assay Lab Chip, and the Caliper Protein Express Reagent kit to perform purity evaluation by Lab on Chip (LoC) Capillary electrophoresis sodium dodecyl sulfate (CE-SDS). Samples and reference standard were diluted to 1 mg/mL in Phosphate Buffered Saline (PBS) pH 7.4. A non-reducing working solution was prepared by diluting 122.5 μL Iodoacetamide (IAM) in 3500 μL of HT Protein Express Sample Buffer to a final concentration of 8.5 mM. Samples were prepared by combining 5 μL of diluted sample with 7 μL of the non-reducing working solution in a microcentrifuge tube, followed by centrifugation at 3000 x g for 1 minute and heating at $70 \pm 2^\circ\text{C}$ for 3 minutes on a heat block. The samples were then cooled to room temperature followed by centrifugation at 3000 x g for 1 minute. The sample preparations were then diluted with 32 μL of water and transferred to a 96 well plate. A blank was prepared using 5 μL of PBS pH 7.4 and the same sample preparation procedure to ensure there are no interfering peaks. See R CE-SDS above for chip preparation.

In the first stability study (Example 11), non-reducing CE-SDS sample preparation was performed without IAM (combining 7 μL of HT Protein Express sample buffer with 5 μL of diluted sample). The method was updated to include IAM prior to the stability study reported in Example 12, to reduce variability in the observed due to artificial changes in purity caused by the interaction of SDS with the sample.

In the second stability study, IAM was added to the non-reducing working solution to decrease variability in purity attributed to the presence of free sulfhydryls caused by the addition of SDS. Sulfhydryls can induce the formation of new species by disulfide bond scrambling observed as new peaks, resulting in artificial decreases in sample purity. IAM is an
5 alkylating agent which can block the sulfhydryls and prevent disulfide bond scrambling.

Results and Discussions

Biophysical testing:

Biophysical testing was performed by DSF and DLS to evaluate the effects of buffer identity,
10 solution pH, and excipients on the stability of IgG1-C-E430G. The formulations evaluated are shown in Table 5. Results obtained from DSF and DLS analysis are shown in Table 8.

Table 8. Biophysical Study - DSF and DLS Results

BS ID	DLS Avg T _{onset} °C	DSF Avg T _{m1} °C	DLS Avg T _{onset2} °C	DSF Avg T _{m2} °C	T _{agg} at SLS 266nm °C	T _{agg} at SLS 473nm °C	DLS Avg Z- Ave. Dia (nm)	DLS Avg. PDI	DLS Avg Pk 1 Mode Dia (nm)	DLS Avg Pk 1 Polydispersity (%)	DLS Modality	DLS Pk 1 % Mass
F1	51.9	57.7	72	82.2	83.7	83.6	12.1	0.4	9.9	29.7	Multimodal	99.99
F2	52.1	57.5	72.2	82.2	78.5	79	12.6	0.1	10.6	20.3	Multimodal	99.92
F3	51.5	57.1	72.5	84.3	81	81.3	11.0	0.5	10.9	34.8	Monomodal	100.00
F4	52.4	59.1	73.8	83.5	83.3	83.2	18.1	0.1	12.1	25.4	Multimodal	99.50
F5	55.2	60.5	74.3	90.3	84.7	84.1	11.9	1.2	13.1	35.6	Multimodal	99.99
F6	47.8	55.1	72.3	83	74.6	72.7	14.7	0.0	10.7	19.5	Multimodal	99.79
F7	48	54.4	71.4	83.8	77.9	78.5	15.0	0.1	10.7	15.4	Multimodal	99.80
F8	48.1	53.7	71.1	82.8	79.7	79.8	10.7	0.6	12.4	28.0	Monomodal	100.00
F9	51.6	56.5	74.3	83.6	75.3	74.8	13.3	0.3	11.5	29.2	Multimodal	99.96
F10	51.7	57.2	73.4	84.5	69.7	66.3	23.6	0.3	13.5	28.7	Multimodal	99.85
F11	52.4	58.2	73.9	84.6	75.2	75.7	11.2	0.2	10.1	18.2	Multimodal	99.99
F12	52.1	57.3	73	83.5	80.2	80.4	10.6	0.1	10.7	11.5	Monomodal	100.00
F13	51.9	56.8	72.7	84.7	81.7	81.8	10.6	0.6	11.0	19.2	Monomodal	100.00
F14	54.6	59.4	74.2	85.9	75.2	78.5	19.4	0.0	12.8	29.8	Multimodal	99.77
F15	55.1	59.6	75.6	86.1	79.1	83.3	11.8	1.4	13.1	36.3	Multimodal	99.95
F16	56.8	61.7	78.1	88.1	75.3	75.2	11.4	0.1	9.9	21.6	Multimodal	100.00

T_m: Melting temperature; T_{onset}: Onset of aggregation; T_{agg}: Aggregation temperature; Avg Z-Ave: Average Z-Average; Dia: diameter; PDI: Polydispersity Index; Avg Pk 1: Average peak 1.

Based on the data obtained from DSF and DLS, in terms of buffer type, acetate provided better thermal stability compared to histidine, as indicated by higher T_{onset} values and higher T_{agg} values. However, the degree of aggregation was significantly higher in acetate formulations, as observed by the increased SLS counts at both 266 nm and 473 nm under elevated temperatures. There was an observed positive correlation with solution pH and both T_{onset} and T_{agg} indicating more thermal stability at higher pH. However, there was also a positive correlation observed between solution pH and SLS counts at 266 nm and 473 nm, indicating lower pH solutions may confer greater colloidal stability for IgG1-C-E430G in solution. Sucrose formulations appeared to generate larger aggregates of IgG1-C-E430G due to the higher pk 1 diameter (*i.e.*, the size of the main peak in DLS analysis) and polydispersity values observed with this excipient.

Select formulations were further screened after being subjected to physical stress screening with freeze-thaw cycles and continuous physical agitation. The addition of a surfactant, PS80, was also screened for the impact it had on IgG1-C-E430G stability.

15 Physical Stress and Surfactant Study:

Twelve formulations were screened to evaluate the effects of solution pH, excipients, and surfactant had on IgG1-C-E430G stability. The formulations screened for physical stress and surfactant study are shown in Table 6. Formulations with arginine were excluded because of the biophysical results observed in the DSF analysis. Results for each analytical assay are presented in the tables below.

Table 9. Physical Stress Study - Appearance Results

Formulation	Condition	Color	Clarity	Particulates
20 mM acetate, 150 mM NaCl, pH 5.5	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	very few particles
	Freeze/Thaw	Slightly yellow	clear liquid	very few particles
20 mM acetate, 250 mM sucrose, pH 5.5	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	very few particles
20 mM Histidine, pH 6.0	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles
20 mM Histidine, 150 mM NaCl, pH 6.0	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles
	Control	Slightly yellow	clear liquid	free of visible particles

20 mM Histidine, 250 mM Sorbitol, pH 6.0	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	very few particles
20 mM Histidine, 250 mM Sucrose, pH 6.0	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles
20 mM acetate, 150 mM NaCl, pH 5.5, 0.04% PS80	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles
20 mM acetate, 250 mM sucrose, pH 5.5, 0.04% PS80	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles, except one strand ¹
20 mM Histidine, pH 6.0, 0.04% PS80	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles
20 mM Histidine, 150 mM NaCl, pH 6.0, 0.04% PS80	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles
20 mM Histidine, 250 mM Sorbitol, pH 6.0, 0.04% PS80	Control	Slightly yellow	clear liquid	free of visible particles
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles
20 mM Histidine, 250 mM Sucrose, pH 6.0, 0.04% PS80	Control	Slightly yellow	clear liquid	free of visible particles, except one strand ¹
	Agitation	Slightly yellow	clear liquid	free of visible particles
	Freeze/Thaw	Slightly yellow	clear liquid	free of visible particles

¹ Possibly a dust particle

Table 10A. Physical Stress Study - DLS Results

Buffer	pH	Excipient	PS80 (%)	Z-average Diameter for Initial Samples (nm)	Freeze-thaw Z-average diameter (nm)	Delta Freeze thaw (nm)	Agitation z-average Diameter (nm)	Initial Avg %Pd	Freeze-thaw Avg %Pd	Agitation Avg %Pd	Initial Avg Peak2 Diameter (nm) (I)	Freeze-thaw Avg Peak2 Diameter (nm) (I)	Agitation Avg Peak2 Diameter (nm) (I)
Acetate	5.5	NaCl	None	13.0	13.1	0.1	12.8	0.1	0.1	0.1	13.2	12.9	13.2
Acetate	5.5	Sucrose	None	11.2	11.1	-0.1	11.2	0.3	0.4	0.3	11.0	11.1	11.6
Histidine	6.0	None	None	8.7	9.0	0.2	8.5	0.2	0.4	0.1	9.0	8.3	8.1
Histidine	6.0	NaCl	None	13.5	14.1	0.6	13.3	0.1	0.4	0.1	13.6	13.6	13.6
Histidine	6.0	Sorbitol	None	15.2	13.1	-2.1	13.4	1.4	1.0	0.8	9.7	9.2	9.4
Histidine	6.0	Sucrose	None	10.7	11.0	0.3	11.0	0.4	0.3	0.3	11.3	11.0	10.7
Acetate	5.5	NaCl	0.04%	13.2	13.1	-0.1	13.1	0.2	0.1	0.1	13.2	12.9	12.9
Acetate	5.5	Sucrose	0.04%	11.3	10.8	-0.5	10.9	0.3	0.4	0.3	11.9	11.6	11.0
Histidine	6.0	None	0.04%	8.6	8.5	-0.1	8.7	0.1	0.1	0.1	9.0	8.5	8.3
Histidine	6.0	NaCl	0.04%	13.9	13.0	-0.9	13.1	0.2	0.1	0.2	13.2	13.2	13.2
Histidine	6.0	Sorbitol	0.04%	10.6	10.6	0.1	10.0	0.1	0.1	0.3	9.7	9.4	9.7
Histidine	6.0	Sucrose	0.04%	7.5	10.2	2.8	10.8	0.3	0.4	0.3	11.3	10.5	9.7

% Pd = Percent Polydispersity

Table 10B. Physical Stress Study - DLS Results

Buffer	pH	Excipient	PS80 (%)	Initial Avg Peak2 %Pd (1)	Freeze-thaw Avg Peak2 %Pd (1)	Agitation Avg Peak2 %Pd (1)	Modality Initial	Modality Freeze-thaw	Modality Agitation
Acetate	5.5	NaCl	None	20.5	17.7	16.5	Monomodal	Monomodal	Monomodal
Acetate	5.5	Sucrose	None	30.5	29.5	31.0	Monomodal	Monomodal	Monomodal
Histidine	6.0	None	None	26.4	31.8	29.2	Monomodal	Monomodal	Monomodal
Histidine	6.0	NaCl	None	18.9	23.8	16.8	Monomodal	Monomodal	Monomodal
Histidine	6.0	Sorbitol	None	22.2	19.4	22.3	Monomodal	Multimodal	Multimodal
Histidine	6.0	Sucrose	None	30.7	26.1	21.3	Monomodal	Monomodal	Monomodal
Acetate	5.5	NaCl	0.04%	19.7	26.3	24.6	Monomodal	Monomodal	Monomodal
Acetate	5.5	Sucrose	0.04%	31.7	29.1	28.2	Monomodal	Monomodal	Monomodal
Histidine	6.0	None	0.04%	23.8	23.0	28.5	Monomodal	Monomodal	Monomodal
Histidine	6.0	NaCl	0.04%	27.2	20.5	16.5	Monomodal	Monomodal	Monomodal
Histidine	6.0	Sorbitol	0.04%	25.9	29.0	20.6	Monomodal	Monomodal	Monomodal
Histidine	6.0	Sucrose	0.04%	28.7	25.5	30.2	Monomodal	Monomodal	Monomodal

Table 11. Physical Stress Study - A280 Results

Formulation Code	Sample	Conc. (mg/mL)
20 mM acetate, 150 mM NaCl, pH 5.5	Control	21.7
	Agitation	21.4
	Freeze/Thaw	21.8
20 mM acetate, 250 mM sucrose, pH 5.5	Control	22.2
	Agitation	21.9
	Freeze/Thaw	21.7
20 mM Histidine, pH 6.0	Control	22.8
	Agitation	22.5
	Freeze/Thaw	22.5
20 mM Histidine, 150 mM NaCl, pH 6.0	Control	21.6
	Agitation	22.2
	Freeze/Thaw	21.9
20 mM Histidine, 250 mM Sorbitol, pH 6.0	Control	21.5
	Agitation	22.2
	Freeze/Thaw	21.6
20 mM Histidine, 250 mM Sucrose, pH 6.0	Control	21.9
	Agitation	21.7
	Freeze/Thaw	21.4
20 mM acetate, 150 mM NaCl, pH 5.5, 0.04% PS80	Control	21.9
	Agitation	22.4
	Freeze/Thaw	22.6
20 mM acetate, 250 mM sucrose, pH 5.5, 0.04% PS80	Control	22.3
	Agitation	22.8
	Freeze/Thaw	22.7
20 mM Histidine, pH 6.0, 0.04% PS80	Control	22.7
	Agitation	22.7
	Freeze/Thaw	22.7
20 mM Histidine, 150 mM NaCl, pH 6.0, 0.04% PS80	Control	22.0
	Agitation	22.5
	Freeze/Thaw	22.5
20 mM Histidine, 250 mM Sorbitol, pH 6.0, 0.04% PS80	Control	22.2
	Agitation	22.0
	Freeze/Thaw	22.2
	Control	22.5
	Agitation	22.8

20 mM Histidine, 250 mM Sucrose, pH 6.0, 0.04% PS80	Freeze/Thaw	22.2
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Table 12. Physical Stress Study - Turbidity Results

Formulation	Sample	A550 (AU)
20 mM acetate, 150 mM NaCl, pH 5.5	Control	0.04244
	Agitation	0.04658
	Freeze/Thaw	0.04404
20 mM acetate, 250 mM sucrose, pH 5.5	Control	0.02345
	Agitation	0.03125
	Freeze/Thaw	0.04969
20 mM Histidine, pH 6.0	Control	0.04515
	Agitation	0.02795
	Freeze/Thaw	0.01916
20 mM Histidine, 150 mM NaCl, pH 6.0	Control	0.04425
	Agitation	0.03002
	Freeze/Thaw	0.03813
20 mM Histidine, 250 mM Sorbitol, pH 6.0	Control	0.03038
	Agitation	0.01559
	Freeze/Thaw	0.03248
20 mM Histidine, 250 mM Sucrose, pH 6.0	Control	0.03673
	Agitation	0.03689
	Freeze/Thaw	0.08891
20 mM acetate, 150 mM NaCl, pH 5.5, 0.04% PS80	Control	0.03743
	Agitation	0.02207
	Freeze/Thaw	0.02036
20 mM acetate, 250 mM sucrose, pH 5.5, 0.04% PS80	Control	0.02019
	Agitation	0.02036
	Freeze/Thaw	0.04208
20 mM Histidine, pH 6.0, 0.04% PS80	Control	0.01594
	Agitation	0.01374
	Freeze/Thaw	0.01612
20 mM Histidine, 150 mM NaCl, pH 6.0, 0.04% PS80	Control	0.02464
	Agitation	0.02275
	Freeze/Thaw	0.02205
20 mM Histidine, 250 mM Sorbitol, pH 6.0, 0.04% PS80	Control	0.02088
	Agitation	0.02585

	Freeze/Thaw	0.02225
20 mM Histidine, 250 mM Sucrose, pH 6.0, 0.04% PS80	Control	0.13415 ¹
	Agitation	0.03337
	Freeze/Thaw	0.03637

¹ Likely an outlier, as the two stressed samples in this formulation had lower A550 values

Table 13. Physical Stress Study - SEC Results

ID	PS80 (%)	Initial SEC purity (%)	Freeze-thaw SEC purity (%)	Agitation SEC purity (%)	Initial SEC HMW (%)	Freeze-thaw SEC HMW (%)	Agitation SEC HMW (%)	Initial SEC LMW (%)	Freeze-thaw SEC LMW (%)	Agitation SEC LMW (%)
20 mM acetate, 150 mM NaCl, pH 5.5	None	99.0	98.7	99.0	0.7	1.1	0.8	0.3	0.2	0.2
20 mM acetate, 250 mM sucrose, pH 5.5	None	99.2	99.4	99.3	0.6	0.4	0.5	0.2	0.1	0.2
20 mM Histidine, pH 6.0	None	99.2	99.5	99.3	0.6	0.4	0.5	0.2	0.1	0.2
20 mM Histidine, 150 mM NaCl, pH 6.0	None	99.1	99.3	99.3	0.6	0.5	0.4	0.3	0.2	0.2
20 mM Histidine, 250 mM Sorbitol, pH 6.0	None	99.2	99.5	99.4	0.5	0.4	0.4	0.3	0.2	0.2
20 mM Histidine, 250 mM Sucrose, pH 6.0	None	99.2	99.5	99.5	0.5	0.4	0.4	0.3	0.2	0.1
20 mM acetate, 150 mM NaCl, pH 5.5, 0.04% PS80	0.04%	99.0	99.1	99.1	0.8	0.7	0.7	0.3	0.2	0.3
20 mM acetate, 250 mM sucrose, pH 5.5, 0.04% PS80	0.04%	99.2	99.5	99.4	0.5	0.4	0.4	0.2	0.1	0.1
20 mM Histidine, pH 6.0, 0.04% PS80	0.04%	99.3	99.4	99.3	0.5	0.4	0.5	0.2	0.2	0.3
20 mM Histidine, 150 mM NaCl, pH 6.0, 0.04% PS80	0.04%	99.3	99.2	99.3	0.5	0.6	0.5	0.2	0.2	0.2
20 mM Histidine, 250 mM Sorbitol, pH 6.0, 0.04% PS80	0.04%	99.3	99.6	99.4	0.4	0.3	0.4	0.3	0.1	0.1
20 mM Histidine, 250 mM Sucrose, pH 6.0, 0.04% PS80	0.04%	99.2	99.5	98.4	0.4	0.3	0.4	0.5	0.1	1.2

In terms of buffer identity, only the 20 mM acetate, 150 mM NaCl at pH 5.5 formulation showed visible particles following exposure to 48 hours of continuous agitation. 20 mM acetate, 150 mM NaCl at pH 5.5, and 20 mM acetate, 250 mM sucrose, at pH 5.5, and 20 mM histidine, 250 mM sorbitol, at pH 6.0 were the only formulations observed to form
5 particulates following five freeze-thaw cycles. However, comparing these formulations to their counterparts containing 0.04% (w/v) PS80, no particulates are observed, which indicated that including PS80 in the formulation of IgG1-C-E430G may help protect against particulate formation.

In terms of excipients, SLS data (Table 8) demonstrated that formulations with charged
10 excipients (NaCl, Arginine) had significantly higher SLS counts at both 266 and 473 nm at elevated temperatures compared to the SLS counts of sugar excipients, indicating sugars provide greater colloidal stability to IgG1-C-E430G.

The 20 mM acetate, 150 mM NaCl at pH 5.5 was the only formulation with an observed decrease in purity after physical stress, after the five freeze-thaw cycles. However, including
15 PS80 in the formulation appears to prevent the growth of aggregates after freeze-thawing, as the purity remains consistent, even after freeze-thawing.

There was an observed change in A_{280} of IgG1-C-E430G following freeze-thaw cycles in formulations with sucrose.

Based on these compiled results, 20 mM histidine, 250 mM sorbitol, 0.04% (w/v) PS80, pH
20 6.0 was selected as the optimal formulation for IgG1-C-E430G.

Stability Study

The stability of the identified formulation, 20mM histidine, 250mM sorbitol, 0.04% PS80, pH 6.0 (Table 7), for IgG1-C-E430G was then assessed at 20 mg/mL over 8 weeks at $5\pm3^{\circ}\text{C}$, $25\pm2^{\circ}\text{C}/60\pm5\%$ RH, and $40\pm2^{\circ}\text{C}/75\pm5\%$ RH storage conditions. The initial samples were
25 tested by appearance, pH, A_{280} , SE-HPLC, icIEF, microchip CE-SDS (reduced and non-reduced). Additional samples were prepared at the initial timepoint and subjected to five freeze-thaw cycles as well as physical agitation (400 RPM for 48 hours) to confirm the results of the physical stress study. The results of selected analyses are shown in the Tables below.

Table 14. Stability Study LoC-NR Results

Timepoint	Condition	% Main Peak	% Front-Side Related Substances/ Impurities ¹	% Back-Side Related Substances/ Impurities	% Total Related Substances/ Impurities
Initial	N/A	95.0	5.0	Not detected (ND)	5.0
	Freeze-Thaw	95.8	4.2	ND	4.2
	Agitation	94.8	5.2	ND	5.2
2 weeks	2-8°C	91.4	8.6	ND	8.6
	25°C/60%RH	91.1	8.9	ND	8.9
	40°C/75%RH	90.2	9.8	ND	9.8
4 weeks	2-8°C	91.2	8.8	ND	8.8
	25°C/60%RH	92.1	7.9	ND	7.9
	40°C/75%RH	91.0	9.0	ND	9.0
8 weeks	2-8°C	88.8	11.2	ND	11.2
	25°C/60%RH	91.2	8.8	ND	8.8
	40°C/75%RH	89.7	10.3	ND	10.3

¹ Variability of front-side related impurities (fragments) likely due to uncontrolled disulfide-bond scrambling catalyzed by free sulfhydryl groups due to lack of alkylating agent in sample preparations.

5 Table 15. Stability Study LoC-R Results

Timepoint	Condition	% Light Chain	% Heavy Chain	% Total Related Substances/ Impurities	% Purity
Initial	N/A	34.5	64.2	1.3	98.7
	Freeze-Thaw	34.5	64.4	1.2	98.8
	Agitation	34.6	64.1	1.4	98.6
2 weeks	2-8°C	33.9	63.9	2.1	97.9
	25°C/60%RH	34.0	63.8	2.2	97.8
	40°C/75%RH	34.5	62.9	2.6	97.4
4 weeks	2-8°C	34.4	63.1	2.5	97.5
	25°C/60%RH	34.4	63.2	2.4	97.6
	40°C/75%RH	34.9	61.9	3.2	96.8
8 weeks	2-8°C	34.6	62.0	3.4	96.6
	25°C/60%RH	34.8	62.4	2.8	97.2
	40°C/75%RH	35.5	61.0	3.5	96.5

Table 16. Stability Study icIEF Results

Timepoint	Condition	% Acidic Variants	% Main Peak	% Basic Variants	pI of Main Peak
Initial	N/A	49.53	50.47	ND	8.81
	Freeze-Thaw	49.01	51.00	ND	8.80
	Agitation	50.58	49.42	ND	8.80
2 weeks	2-8°C	47.32	52.68	ND	8.81
	25°C/60%RH	48.49	51.51	ND	8.81
	40°C/75%RH	57.45	42.55	ND	8.81
4 weeks	2-8°C	50.55	49.45	ND	8.80
	25°C/60%RH	50.81	49.19	ND	8.80
	40°C/75%RH	65.58	34.42	ND	8.80
8 weeks	2-8°C	51.47	48.53	ND	8.82
	25°C/60%RH	55.26	44.74	ND	8.82
	40°C/75%RH	72.91	27.09	ND	8.82

Based on the data obtained from the stability study, there was no impact to appearance or particle content of the solutions, regardless of storage condition. There appeared to be no impact to solution pH or A_{280} , regardless of storage condition over the duration of the study, except for the A_{280} at $40\pm 2^\circ\text{C}/70\pm 5\%\text{RH}$, which was observed to increase over time. This increase in A_{280} can likely be attributed to evaporation at the higher storage temperature.

SE-HPLC results showed that there was no impact to IgG1-C-E430G purity following physical stress of freeze-thaw cycles or 48 hours or continuous agitation. Each condition showed similar trends in terms of monomer purity over the course of the study, with decreases in purity at each timepoint, however, the effects on purity were more significant in the $40\pm 2^\circ\text{C}/70\pm 5\%\text{RH}$ accelerated storage condition. Under temperature stress conditions, both LMW and HMW IgG1-C-E430G species increased over time. For the $5\pm 3^\circ\text{C}$ and $25\pm 2^\circ\text{C}/60\pm 5\%\text{RH}$ condition, only an increase in %LMW was observed through 8 weeks.

No significant changes in purity of IgG1-C-E430G by LoC-NR analysis were observed. Under reducing conditions, the IgG1-C-E430G purity decreased and increased fragmentation was observed for all conditions up to 8 weeks, though the changes were more pronounced under accelerated storage conditions.

icIEF results indicated there were no changes to charge heterogeneity of IgG1-C-E430G at the nominal condition ($5\pm 3^{\circ}\text{C}$), however, there were changes at both accelerated storage conditions ($25\pm 2^{\circ}\text{C}/60\pm 5\%\text{RH}$ and $40\pm 2^{\circ}\text{C}/70\pm 5\%\text{RH}$). An increase in % acidic variants was observed at each successive timepoint, likely a result of protein deamidation under
5 accelerated storage conditions. There was no change in pI of the main peak regardless of storage condition across the entire study.

Conclusions

From the results obtained from the biophysical study, physical stress study, and surfactant study, 20 mM histidine, 250 mM sorbitol, 0.04% PS80, at pH 6.0 (Table 7), was identified as
10 the optimal formulation for IgG1-C-E430G for evaluation of product stability at nominal and accelerated storage conditions.

The initial biophysical study evaluated the effects of buffer type, excipients, and pH on IgG1-C-E430G thermal and conformational stability. Based on the results obtained from DSF and DLS, acetate appeared to provide better thermal stability compared to histidine, with higher
15 observed T_{onset} and T_{agg} values, however the degree of aggregation was significantly higher in acetate formulations as observed by the SLS counts at 266 and 473 nm at elevated temperatures. Acetate formulations appeared to generally have lower z-avg particle diameter, except for the 20 mM histidine, 250 mM sorbitol at pH 5.5. However, acetate formulations also appeared to have higher avg pk 1 polydispersity % compared to histidine,
20 indicating larger aggregates present in the acetate formulations.

In terms of solution pH, there was a positive correlation observed between histidine formulations and T_{agg} values, indicating higher solution pH may increase the temperature for the onset of protein aggregation. However, SLS counts demonstrated that the degree of aggregation is higher in high pH solutions, indicating lower pH formulations have greater
25 colloidal stability. DLS results showed pk 1 polydispersity was highest in the high pH (6.5) formulation, indicating higher pH solutions may potentially have higher population of oligomers such as dimers and trimers of IgG1-C-E430G.

In terms of excipients, SLS data demonstrated that formulations with charged excipients (NaCl, Arginine) had significantly higher SLS counts at both 266 and 473 nm at elevated
30 temperatures compared to the SLS counts of sugar excipients, indicating sugars provide greater colloidal stability to IgG1-C-E430G. Sucrose formulations generally had the highest pk 1 diameter and pk 1 polydispersity, indicating these formulations contained a higher population of small oligomers. Although z-average particle diameter was larger in some formulations due to the presence of a secondary population (multimodal), the overall mass %

of peak 1 in these samples is very high (>99%), which indicated the overall contribution of the secondary diameter is minimal.

The six best formulations selected from the biophysical study were further evaluated in the presence of physical stressors (48 hours agitation at 400 RPM and five freeze-thaw cycles).

- 5 These formulations were also evaluated with PS80 added, to determine if the surfactant provided any benefit to IgG1-C-E430G formulation. Appearance testing showed no differences regarding color or clarity after exposure to the stressed conditions. However, formation of particulates was observed in the 20 mM acetate, 150 mM NaCl at pH 5.5 formulation after being exposed to 48 hours of agitation. 20 mM acetate, 150 mM NaCl, at
- 10 pH 5.5, 20 mM acetate, 250 mM sucrose at pH 5.5, and 20 mM histidine, 250 mM sorbitol at pH 6.0 were the only formulations to show particulates following five freeze-thaw cycles. Including PS80 in the formulation appeared to help prevent the formation of particulates following freeze-thaw or agitation physical stresses for all samples. From the DLS results, it was observed that formulations with NaCl exhibited higher z-average particle diameter.
- 15 There appeared to be no differences in z-average particle diameter between formulations with and without PS80, except for the 20 mM histidine and sugar (250 mM sorbitol and 250 mM sucrose) formulations where a significant decrease in z-average particle diameter was observed with 0.04% (w/v) PS80 included in the formulation. This indicated that PS80 will potentially help prevent aggregation of IgG1-C-E430G in histidine and sugar formulations.
- 20 SE-HPLC results showed that acetate with NaCl saw a decrease in purity following the freeze-thaw cycles, which was attributed to a growth in % HMW species, however this drop in purity was mitigated by adding PS80 into the solution, with no drop in purity observed following physical stressors. Based on the generated DLS, appearance, and SE-HPLC data, it indicated that 20 mM histidine, 250 mM sorbitol, 0.04% PS80, at pH 6.0 was the optimal formulation
- 25 to move into stability.

- Finally, a stability study was performed evaluating the optimal IgG1-C-E430G formulation (20 mM histidine, 250 mM sorbitol, 0.04% PS80, at pH 6.0) at 20 mg/mL. Samples were incubated at a nominal condition ($5\pm 3^{\circ}\text{C}$), and two accelerated conditions ($25\pm 2^{\circ}\text{C}/60\pm 5\%\text{RH}$ and $40\pm 2^{\circ}\text{C}/70\pm 5\%\text{RH}$) for eight weeks. After incubation, samples
- 30 were tested by appearance, pH, A_{280} , SE-HPLC, LoC, and icIEF. The freeze-thaw cycles, and physical agitation was evaluated at the initial timepoint for this stability study.

Appearance, pH, and A_{280} testing showed no differences between the storage condition, and no visible particles were observed up to 8 weeks.

- SE-HPLC results showed decreases in monomer purity for each timepoint at each condition,
- 35 although the impact was most significant in the accelerated conditions. The accelerated

condition ($40\pm 2^{\circ}\text{C}/70\pm 5\%\text{RH}$) was also the only sample to show increases in % HMW species, while the other two conditions showed increases in % LMW species only.

From the LoC-NR results, an initial drop off in purity was observed in each sample by the 2-week timepoint, however, the impurities may reach an equilibration and not increase further.

5 However, further timepoints would need to be analyzed to confirm this hypothesis.

icIEF results showed that % main peak, % acidic variants, and % basic variants are similar across the study for the nominal condition ($5\pm 3^{\circ}\text{C}$). However, at accelerated conditions ($25\pm 2^{\circ}\text{C}/60\pm 5\%\text{RH}$ and $40\pm 2^{\circ}\text{C}/70\pm 5\%\text{RH}$), degradation of the % main peak led to increases in % acidic variants, indicating potential deamination of IgG1-C-E430G at higher
10 temperatures.

Example 12 – Antibody formulation - second stability study

A second stability study of 20 mg/mL IgG1-C-E430G formulation (in 20mM Histidine, 250mM sorbitol, pH 6.0, 0.04% PS80) was performed.

Methods

15 Except as provided below, the methods used are described in Example 11.

Micro-Flow Imaging (MFI):

This method utilizes the ProteinSimple micro-flow imaging (MFI) microscope to collect images of and allow characterization of sub-visible particles. Sample analysis is performed by transferring 1mL of each sample into a well on a 96-well Eppendorf Deepwell plate. The
20 instrument method is created using the MFI View System Software (MVSS). Images for all particles with an equivalent circular diameter (ECD) ≥ 1 and ≤ 100 micron are collected by the instrument.

Following sample analysis, the MVSS software is used to apply morphological filters to the collected images and grouping particles into populations based on common characteristics.

25 Images are filtered by ECD into the following groups: ECD ≥ 2 micron, ECD ≥ 5 micron, ECD ≥ 10 micron, and ≥ 25 micron. The aspect ratio morphological filter is applied with a cutoff of ≥ 0.85 to the ≥ 5 -micron population, which identifies images containing circular and non-circular particles. Non-circular particles ($\text{AR} < 0.85$) tend to be proteinaceous in origin, whereas circular particles ($\text{AR} \geq 0.85$) tend to be comprised of air bubbles and silicone oil.

The images containing circular particles can then be characterized as air bubbles or to silicone particles based on intensity min which the MVSS software defines as the intensity of the lightest pixel within a particle. The filters are used to group round particles with an intensity min > 75 as air bubbles, and particles with an intensity min ≥ 75 and ≤ 300 as

5 Silicone particles. Silicone and circular fraction and can then be determined by dividing the number of particles determined to be Silicone or circular by the total number of particles with the same ECD.

Results for this method are reported as particle counts per mL for desired characterization group determined by the morphological features. The results from MFI can be used to assess

10 the stability of a formulated antibody via observation of changes particle counts over the course of a stability study. MFI is only used as an indirect characterization of a particle, not a direct measurement of the particle's composition.

Results

The results from the second stability study are shown in the tables below.

15 Table 17. Appearance

Timepoint	Condition	Color	Clarity	Particulates
Initial	Control	Slightly Yellow	Clear Liquid	Free of visible particulates
	Agitation	Slightly Yellow	Clear Liquid	Free of visible particulates
	Agitation Control	Slightly Yellow	Clear Liquid	Free of visible particulates
	Freeze/Thaw	Slightly Yellow	Clear Liquid	Free of visible particulates
2 weeks	2-8°C	Very Slightly Yellow	Clear Liquid	Free of visible particulates
	25°C/60%RH	Very Slightly Yellow	Clear Liquid	Free of visible particulates
	40°C/75%RH	Very Slightly Yellow	Clear Liquid	Free of visible particulates
4 weeks	2-8°C	Very Slightly Yellow	Clear Liquid	Very few visible particulates

	25°C/60%RH	Very Slightly Yellow	Clear Liquid	Very few visible particulates
	40°C/75%RH	Very Slightly Yellow	Clear Liquid	Very few visible particulates
8 weeks	2-8°C	Very Slightly Yellow	Clear Liquid	Very few visible particulates
	25°C/60%RH	Very Slightly Yellow	Clear Liquid	Very few visible particulates
	40°C/75%RH	Slightly Yellow	Clear Liquid	Very few visible particulates
12 weeks	2-8°C	Very Slightly Yellow	Clear Liquid	Very few visible particulates
	25°C/60%RH	Very Slightly Yellow	Clear Liquid	Some visible particulates
	40°C/75%RH	Slightly Yellow	Clear Liquid	Very few visible particulates

Table 18. pH

Timepoint	Condition	pH
Initial	N/A	6,0
	Freeze/Thaw	6,0
	Agitation	6,0
	Agitation Control	6,0
2 weeks	2-8°C	6,0
	25°C/60%RH	5,9
	40°C/75%RH	5,9
4 weeks	2-8°C	5,9
	25°C/60%RH	5,9
	40°C/75%RH	5,9
8 weeks	2-8°C	6,0
	25°C/60%RH	6,0
	40°C/75%RH	6,0

12 weeks	2-8°C	5,9
	25°C/60%RH	5,9
	40°C/75%RH	5,9

Table 19. Absorbance at 280 nm (A_{280})

Timepoint	Condition	Concentration (mg/mL)
Initial	N/A	20,6
	Freeze-Thaw	20,9
	Agitation	21,0
	Agitation Control	21,1
2 weeks	2-8°C	21,2
	25°C/60%RH	21,2
	40°C/75%RH	20,7
4 weeks	2-8°C	20,9
	25°C/60%RH	21,4
	40°C/75%RH	21,4
8 weeks	2-8°C	21,4
	25°C/60%RH	20,4
	40°C/75%RH	21,4
12 weeks	2-8°C	22,2
	25°C/60%RH	21,9
	40°C/75%RH	21,5

Table 20. Size-Exclusion Chromatography (SEC)

Timepoint	Condition	% HMW	% Monomer	% LMW	Total Impurities	Total Area (mAU*s)
Initial	N/A	1,3	98,6	0,1	1,4	4286

	Freeze-Thaw	1,3	98,6	0,2	1,4	4290
	Agitation	1,3	98,5	0,2	1,5	4331
	Agitation Control	1,3	98,5	0,2	1,5	4228
2 weeks	2-8°C	1,4	98,5	0,1	1,5	4093
	25°C/60%RH	1,5	98,3	0,2	1,7	4253
	40°C/75%RH	2,4	97,0	0,5	3,0	4115
4 weeks	2-8°C	1,4	98,5	0,1	1,5	4307
	25°C/60%RH	1,6	98,2	0,2	1,8	4336
	40°C/75%RH	2,5	96,5	1,0	3,5	4112
8 weeks	2-8°C	1,7	98,3	ND	1,7	4406
	25°C/60%RH	1,8	97,8	0,3	2,2	4196
	40°C/75%RH	3,8	94,1	2,1	5,9	4064
12 weeks	2-8°C	1,5	98,4	0,1	1,6	4204
	25°C/60%RH	2,0	97,6	0,5	2,4	4170
	40°C/75%RH	3,9	92,7	3,4	7,3	3890

Table 21. LoC-NR

Timepoint	Condition	% Main Peak	% Front-Side Related Substances/ Impurities	% Back-Side Related Substances/ Impurities	% Total Related Substances/ Impurities
Initial	N/A	92,4	7,6	ND	7,6
	Freeze/Thaw	92,2	7,8	ND	7,8
	Agitation	92,2	7,8	ND	7,8
	Agitation Control	92,2	7,8	0,1	7,8
2 weeks	2-8°C	90,8	9,0	0,2	9,2
	25°C/60%RH	90,1	9,6	0,2	9,9
	40°C/75%RH	88,5	11,2	0,3	11,5

4 weeks	2-8°C	89,9	9,9	0,2	10,1
	25°C/60%RH	89,1	10,8	0,2	10,9
	40°C/75%RH	86,6	13,2	0,2	13,4
8 weeks	2-8°C	89,7	10,1	0,2	10,3
	25°C/60%RH	89,1	10,9	ND	10,9
	40°C/75%RH	82,3	17,5	0,2	17,7
12 weeks	2-8°C	94,7	5,3	ND	5,3
	25°C/60%RH	94,1	5,9	ND	5,9
	40°C/75%RH	87,7	12,3	ND	12,3

Table 22. LoC-R

Timepoint	Condition	% Light Chain	NGHC %	% Heavy Chain	% Total Related Substances/ Impurities	% Purity
Initial	N/A	34,7	0,0	64,6	0,7	99,3
	Freeze-Thaw	34,7	0,0	64,6	0,7	99,3
	Agitation	34,6	0,1	64,6	0,8	99,2
	Agitation Control	34,5	0,1	64,8	0,7	99,3
2 weeks	2-8°C	33,9	0,0	65,2	0,9	99,1
	25°C/60%RH	34,2	0,0	64,7	1,1	98,9
	40°C/75%RH	34,6	0,1	63,8	1,6	98,4
4 weeks	2-8°C	33,6	0,1	66,0	0,5	99,5
	25°C/60%RH	34,0	0,1	65,4	0,6	99,4
	40°C/75%RH	34,5	0,1	64,2	1,2	98,8
8 weeks	2-8°C	33,8	0,0	64,9	1,3	98,7
	25°C/60%RH	33,7	0,1	64,3	2,0	98,0
	40°C/75%RH	34,8	0,8	60,2	5,0	95,0

12 weeks	2-8°C	33,6	0,3	65,3	1,0	99,0
	25°C/60%RH	33,8	0,3	64,4	1,8	98,2
	40°C/75%RH	34,1	0,9	58,4	7,3	92,6

Table 23. MFI Summary

Timepoint	Condition	Counts/mL						
		≥ 2 μm	≥ 5 μm	≥ 10 μm	≥ 25 μm	≥ 5 μm, AR ≥ 0.85	≥ 5 μm, AR ≤ 0.85	Circular Fraction, ≥ 5 μm
Initial	N/A	7133	860	67	6	491	369	0,57
	Freeze-Thaw	14629	2071	287	31	613	1458	0,30
	Agitation	5667	514	71	8	155	359	0,30
	Agitation Control	10503	1292	166	21	558	734	0,43
2 weeks	2-8°C	1685	243	14	2	171	73	0,65
	25°C/60%RH	4762	1102	274	18	112	990	0,06
	40°C/75%RH	3998	695	28	2	93	602	0,10
4 weeks	2-8°C	19602	2262	335	27	489	1772	0,22
	25°C/60%RH	10461	1625	220	4	141	1483	0,09
	40°C/75%RH	9226	1149	130	13	117	1032	0,10
8 weeks	2-8°C	3458	619	113	17	218	401	0,35
	25°C/60%RH	6879	1276	161	6	166	1110	0,13
	40°C/75%RH	8652	2912	455	8	206	2706	0,07
12 weeks	2-8°C	2051	166	21	4	88	78	0,53
	25°C/60%RH	13053	2504	552	50	147	2357	0,06
	40°C/75%RH	19035	4257	277	29	250	4007	0,06

Table 24. icIEF

Timepoint	Condition	% Acidic Variants	% Main Peak	% Basic Variants	pI
Initial	N/A	49,4	45,7	4,9	8,8
	Freeze-Thaw	50,1	45,2	4,7	8,8
	Agitation	50,1	45,2	4,7	8,8
	Agitation Control	50,1	45,3	4,6	8,8
2 weeks	2-8°C	50,4	44,3	5,3	8,8
	25°C/60%RH	56,5	39,2	4,3	8,8
	40°C/75%RH	63,0	33,1	4,0	8,8
4 weeks	2-8°C	52,0	43,3	4,7	8,8
	25°C/60%RH	59,0	37,2	3,8	8,8
	40°C/75%RH	69,2	28,1	2,7	8,9
8 weeks	2-8°C	54,0	40,9	5,1	8,8
	25°C/60%RH	65,2	31,7	3,1	8,8
	40°C/75%RH	78,9	19,0	2,1	8,8
12 weeks	2-8°C	54,1	41,2	4,6	8,8
	25°C/60%RH	64,9	31,5	3,6	8,8
	40°C/75%RH	84,3	13,8	1,8	8,8

Example 13 – Binding affinity determined using Biolayer Interferometry

Target binding affinity of human CD38-specific antibodies IgG1-C-E430G and IgG1-B was
5 determined by label-free biolayer interferometry (BLI) on an Octet HTX instrument
(ForteBio). Experiments were carried out while shaking at 1,000 RPM at 30°C.

Anti-Human IgG Fc Capture (AHC) biosensors (ForteBio, cat. no. 18-5060) were pre-
conditioned by exposure to 10 mM glycine buffer pH 1.7 (Riedel-de Haën, cat. no. 15227) for
5 s, followed by neutralization in Sample Diluent (ForteBio, cat. no. 18-1104) for 5 s; both
10 steps were repeated 5 times. Next, AHC sensors were loaded with antibody (1 µg/mL in
Sample Diluent) for 600 s. After a baseline measurement in Sample Diluent (100 s), the

association (200 s) and dissociation (1,000 s) of recombinant His-CD38 (Genmab) was determined using a concentration range of 0.78 - 50 nM (0.02 – 1.53 µg/ml) with 2-fold dilution steps in Sample Diluent. The theoretical molecular mass of His-CD38 based on the amino acid sequence (30.5 kDa) was used for calculations. For each antibody a reference sensor was used, which was incubated with Sample Diluent instead of antigen.

Data were acquired using Data Acquisition Software v9.0.0.49d (ForteBio) and analyzed with Data Analysis Software v9.0.0.14 (ForteBio). Data traces were corrected per antibody by subtraction of the reference sensor. The Y-axis was aligned to the last 10 s of the baseline and Interstep Correction alignment to dissociation as well as Savitzky-Golay filtering were applied. Data traces with a response < 0.04 nm were excluded from analysis. The data was fitted with the 1:1 model using a window of interest for the association and dissociation times set at 200 s and 1,000 s, respectively.

" K_D " (M) refers to the dissociation equilibrium constant of the antibody-antigen interaction and is obtained by dividing k_d by k_a . " k_d " (sec^{-1}) refers to the dissociation rate constant of the antibody-antigen interaction. This is sometimes also referred to as the k_{off} value or off-rate. " k_a " ($\text{M}^{-1} \times \text{sec}^{-1}$) refers to the association rate constant of the antibody-antigen interaction. This is sometimes also referred to as the k_{on} value or on-rate.

Table 25 shows the results of three independent experiments. The average affinity (dissociation equilibrium constant, K_D) of IgG1-C-E430G for His-CD38 was 1.1 ± 0.17 nM. The affinity of IgG1-B for His-CD38 was 3.9 ± 0.48 nM.

Table 25. Binding affinity of CD38 antibodies for His-CD38

Antibody	Experiment	K_D (M)	On-rate k_a (1/Ms)	Off-rate k_d (1/s)
IgG1-C-E430G	1	1.0E-09	3.8E+05	4.0E-04
	2	9.5E-10	4.3E+05	4.1E-04
	3	1.3E-09	4.1E+05	5.2E-04
	Average	1.1E-09	4.1E+05	4.4E-04
	STDEV	1.7E-10	2.4E+04	6.5E-05
IgG1-B	1	4.2E-09	2.3E+05	9.5E-04
	2	3.4E-09	2.9E+05	9.8E-04
	3	4.2E-09	2.5E+05	1.1E-03
	Average	3.9E-09	2.6E+05	9.9E-04
	STDEV	4.8E-10	3.3E+04	5.4E-05

Example 14 – Antibody formulation – further stability studies

Further stability studies were carried out on different batches A and B comprising 20 mg/ml IgG1-C-E430G, formulated in 20 mM histidine, 250 mM sorbitol, 0.04% PS80, pH 6.0.

Results are listed in the tables below and include different storage temperatures for up to 6 months. Parameters assessed were Appearance, Color, Opalescence, Sub-visible particulates, CE-SDS (red) (R CE-SDS), CE-SDS (non-red) (NR CE_SDS), icIEF, SE-HPLC, ELISA, Potency, A280 and pH, which were assessed using methods as described above in examples 11 and 12 and as further described below. The results below confirm that IgG1-C-E430G is considered stable in the formulation and suitable for medical use.

10 Color and opalescence was assessed by visual inspection according to the standardized methods described in the pharmacopeias (European pharmacopeia (Ph.Eur.) and Japanese pharmacopeia (JP)). Color was assessed up against color standards such as 'yellow' (Y), 'brown'(B) or 'brown-yellow' (BY) in increments of 1 to 7 (1 being the most concentrated solution). Opalescence was assessed up against a series of reference suspensions (RSI, RSII, 15 RSIII and RSIV; the latter being the most opalescent solution).

Sub-visible particulates were assessed by analysing the light obscuration caused by free-floating sub-visible particles in accordance to the standardized method described in the pharmacopeias (United States pharmacopeia (USP), European pharmacopeia (Ph.Eur.) and Japanese pharmacopeia (JP). The sub-visible particle count measured after pooling of 20 several vials. The result is calculated and presented as particles per vial.

Potency was determined by Complement dependent cytotoxicity (CDC) assessed using the CellTiter-Glo readout system. This method is based on catalyzation of Luciferin into light emitting oxyluciferin in the presence of ATP, which is a measure for viable cells. In brief, Daudi target cells were thawed and seeded 10^5 cells/well in white opaque 96 well 25 culture plates in assay medium and rested at room temperature (RT) for 60 minutes. Serial dilution of HexaBody-CD38 are prepared in assay medium in separate dilution plates, added to the cells and incubated for 30 min at RT. Pooled Normal Human Serum was added as a source of complement effector proteins in a final concentration of 25% per well and incubated for 30 min at RT. Plates were then wrapped in transparent foil and incubated for 2.5 hours at 30 37°C, 5% CO₂. After equilibration to RT for 30 minutes, CellTiter-Glo reagent was added. Plates were shaken for 30 sec at 400 rpm, incubated for 10 min at RT and luminescence was measured using a multiplate reader.

CD38 binding was determined by ELISA as follows. Binding to the target antigen was 35 assessed using a ligand binding ELISA. The target antigen (HIS-CD38) was diluted in PBS

coated in Nunc Maxisorp ELISA plates and incubated for 16-20hr at 4°C. Assay plates were washed three times using ELISA washers with PBS-Tween-20 (0.05%) between every step. Assay plates were blocked with PBS-T and subsequently incubated for 60 minutes at a temperature-controlled shaker at 25°C and 300rpm. A serial dilution of the samples prepared in PBS-T were added to the plates and incubated for 90 minutes at a temperature-controlled shaker at 25°C and 300rpm. After washing, the in PBS-T pre-diluted Goat anti-Human IgG Fcy-HRP was added and incubated for 60 minutes at a temperature-controlled shaker at 25°C and 300rpm. For detection, after washing the plate, ABTS was added to the assay plate and subsequently incubated for 30 minutes at a temperature-controlled shaker at 25°C and 300rpm. 2% Oxalic acid was added (no washing) to stop the enzymatic reaction. Readout and analysis were performed at 405nm.

Table 26. Stability Data for IgG1-C-E430G, Batch no. A, Storage Temperature 5 ± 3°C – UPRIGHT

Test	Test Parameter	Time Point (months)		
		0	3	6
Appearance	Appearance of solution	Slightly yellow solution, clear, free of visible particles	Conforms	Conforms
Color	Degree of coloration	≤ Y6	≤ Y7	≤ Y6
Opalescence	Degree of opalescence	≤ RSII	≤ RSI	≤ RSI
Sub-visible particulates	Number particles per vial: ≥ 25 µm ≥ 10 µm ≥ 5 µm ≥ 2 µm	0 25 309 1816	13 52 919 8557	367 418 1417 8557
CE-SDS (red)	IgG as HC + LC	99.1%	99.0%	98.9%
CE-SDS (non-red)	Intact IgG Comparison to reference standard	91.5% CR	91.4% CR	91.3% CR
icIEF	% acidic isoforms % main peak % basic isoforms ¹	29.8% 67.0% < LOQ	29.8% 66.9% < LOQ	29.4% 67.4% 3.2%*
SE-HPLC	% monomer	98.6%	98.6%	98.7% 1.3%

	% HMWS % LMWS ²	1.4% < LOQ	1.4% < LOQ	<LOQ
ELISA	% relative to reference standard	102%	103%	105%
Potency	% relative to reference standard	99%	86%	113%
A280	Protein concentration	20.2 mg/mL	20.2 mg/mL	20.2 mg/mL
pH	pH	6.0	6.0	6.0
HC: Heavy chain. HMWS: High molecular weight species. LC: Light chain. LOQ: Limit of Quantitation. LMWS: Low molecular weight species. RS: Reference suspension. Y: Yellow. CR: Compares to reference standard.				
1.	LOQ is 4.9%.			
2.	LOQ is 0.1%.			

Table 27. Stability Data for IgG1-C-E430G, Batch no. A, Storage Temperature 5 ± 3°C – INVERTED.

Test	Test Parameter	Time Point (months)		
		0	3	6
Appearance	Appearance of solution	Slightly yellow solution, clear, free of visible particles	Conforms	≤ Y6
Color	Degree of coloration	≤ Y6	≤ Y7	≤ Y6
Opalescence	Degree of opalescence	≤ RSII	≤ RSI	≤ RSI
Sub-visible particulates	Number particles per vial: ≥ 25 µm ≥ 10 µm ≥ 5 µm ≥ 2 µm	0 25 309 1816	160 194 1022 7438	33 82 1031 7266
CE-SDS (red)	IgG as HC + LC	99.1%	98.7%	98.9%
CE-SDS (non-red)	Intact IgG Comparison to reference standard	91.5% CR	91.6% CR	91.1% CR
icIEF	% acidic isoforms % main peak	29.8% 67.0%	31.2% 65.5%	29.5% 67.4%

Test	Test Parameter	Time Point (months)		
		0	3	6
	% basic isoforms ¹	< LOQ	< LOQ	3.1%*
SE-HPLC	% monomer	98.6%	98.6%	98.7%
	% HMWS	1.4%	1.4%	1.3%
	% LMWS ²	< LOQ	< LOQ	< LOQ
ELISA	% relative to reference standard	102%	105%	107%
Potency	% relative to reference standard	99%	101%	103%
A280	Protein concentration	20.2 mg/mL	20.2 mg/mL	20.2 mg/mL
pH	pH	6.0	6.0	6.0
HC: Heavy chain. HMWS: High molecular weight species. LC: Light chain. LOQ: Limit of Quantitation. LMWS: Low molecular weight species. RS: Reference suspension. Y: Yellow. CR: Compares to reference standard.				
1.	LOQ is 4.9%.			
2.	LOQ is 0.1%.			

Table 28. Stability Data for IgG1-C-E430G, Batch no. A, Storage Temperature $25 \pm 2^\circ\text{C}$ / $60 \pm 5\%$ RH

Test	Test Parameter	Time Point (months)			
		0	2	3	6
Appearance	Appearance of solution	Slightly yellow solution, clear, free of visible particles	Conforms	Conforms	Conforms
Color	Degree of coloration	$\leq$ Y6	$\leq$ Y7	$\leq$ Y7	$\leq$ Y6
Opalescence	Degree of opalescence	$\leq$ RSII	$\leq$ RSI	$\leq$ RSI	$\leq$ RSI
Sub-visible particulates	Number particles per vial:				
	$\geq 25 \mu\text{m}$	0	84	10	6
	$\geq 10 \mu\text{m}$	25	198	72	126
	$\geq 5 \mu\text{m}$	309	1515	1129	2248
	$\geq 2 \mu\text{m}$	1816	7572	7782	33908

Test	Test Parameter	Time Point (months)			
		0	2	3	6
CE-SDS (red)	IgG as HC + LC	99.1%	98.5%	98.5%	97.6%
CE-SDS (non-red)	Intact IgG Comparison to reference standard	91.5% CR	90.9% CR	90.7% CR	89.6% CR
icIEF	% acidic isoforms	29.8%	36.8%	38.1%	45.0%
	% main peak	67.0%	60.2%	59.1%	52.1%
	% basic isoforms ¹	< LOQ	< LOQ	< LOQ	2.9%*
SE-HPLC	% monomer	98.6%	98.6%	98.5%	98.3%
	% HMWS	1.4%	1.3%	1.3%	1.3%
	% LMWS ²	< LOQ	0.1%	0.2%	0.4%
ELISA	% relative to reference standard	102%	107%	105%	104%
Potency	% relative to reference standard	99%	89%	84%	113%
A280	Protein concentration (mg/mL)	20.2 mg/mL	20.3 mg/mL	20.1 mg/mL	20.2 mg/mL
pH	pH	6.0	5.9 ³	6.1	6.0
HC: Heavy chain. HMWS: High molecular weight species. LC: Light chain. LOQ: Limit of Quantitation. LMWS: Low molecular weight species. RS: Reference suspension. Y: Yellow. CR: Compares to reference standard.					
1.	LOQ is 4.9%.				
2.	LOQ is 0.1%.				
3.	Retrospective pH testing representative of TP=2m.				

Table 29. Stability Data for IgG1-C-E430G, Batch no.B, Storage Temperature $\leq -65^{\circ}\text{C}$

Test	Test Parameter	Time Point (months)			
		0	1	2	3
Color	Degree of coloration	BY6 – BY7	BY7	B7 – B8	BY7
Opalescence	Degree of opalescence	RSI	RSI – RSII	RSI	RSI – RSII
CE-SDS (red)	IgG as HC + LC	98.5%	99.0%	99.0%	98.8%
CE-SDS (non-red)	Intact IgG	91.3%	91.1%	91.2%	91.9%
icIEF	% acidic isoforms	27.0%	26.6%	30.5%	30.4%
	% main peak	70.3%	70.8%	66.4%	66.1%
	% basic isoforms ¹	< LOQ	< LOQ	< LOQ	< LOQ
SE-HPLC	% monomer	98.6%	98.7%	98.7%	98.6%
	% HMWS	1.4%	1.3%	1.3%	1.4%
	% LMWS ²	< LOQ	< LOQ	< LOQ	< LOQ
ELISA	% relative to reference standard	93%	103%	113%	99%
Potency	% relative to reference standard	NT ³	NT ³	106%	95%
A280	Protein concentration (mg/mL)	20.2 mg/mL	20.5 mg/mL	20.4 mg/mL	20.1 mg/mL
pH	pH	6.0	6.0	5.9	6.0
HC: Heavy chain. HMWS: High molecular weight species. LC: Light chain. LMWS: Low molecular weight species. NT: Not tested. RS: Reference suspension. BY: Brown-yellow. B: Brown.					
1.	LOQ is 4.9%.				
2.	LOQ is 0.1%.				
3.	No potency testing at TP=0m and TP=1m due to ongoing validation of potency assay.				

Table 30. Stability Data for IgG1-C-E430G, Batch noB, Storage Temperature $5 \pm 3^{\circ}\text{C}$

Test	Test Parameter	Time Point (months)			
		0	1	2	3
Color	Degree of coloration	BY6 – BY7	BY7	B7	BY7
Opalescence	Degree of opalescence	RSI	RSI – RSII	RSI	RSI – RSII
CE-SDS (red)	IgG as HC + LC	98.5%	98.9%	99.0%	98.7%
CE-SDS (non-red)	Intact IgG	91.3%	91.0%	91.1%	91.8%
icIEF	% acidic isoforms	27.0%	27.0%	30.3%	29.9%
	% main peak	70.3%	70.3%	66.5%	66.0%
	% basic isoforms ¹	< LOQ	< LOQ	< LOQ	< LOQ
SE-HPLC	% monomer	98.6%	98.7%	98.7%	98.6%
	% HMWS	1.4%	1.3%	1.3%	1.4%
	% LMWS ²	< LOQ	< LOQ	< LOQ	< LOQ
ELISA	% relative to reference standard	93%	108%	113%	102%
Potency	% relative to reference standard	NT ³	90%	95%	89%
A280	Protein concentration (mg/mL)	20.2 mg/mL	20.5 mg/mL	20.4 mg/mL	20.1 mg/mL
pH	pH	6.0	6.0	5.9	6.0
HC: Heavy chain. HMWS: High molecular weight species. LC: Light chain. LMWS: Low molecular weight species. RS: Reference suspension. BY: Brown-yellow. B: Brown.					
1.	LOQ is 4.9%.				
2.	LOQ is 0.1%.				
3.	No potency testing at TP=0m due to ongoing validation of potency assay.				

Table 31. Stability Data for IgG1-C-E430G, Batch no. B, Storage Temperature $25 \pm 2^\circ\text{C}$ / $60 \pm 5\%$ RH

Test	Test Parameter	Time Point (months)			
		0	1	2	3
Color	Degree of coloration	BY6 – BY7	BY7	B7	BY7
Opalescence	Degree of opalescence	RSI	RSI – RSII	RSI	RSI – RSII
CE-SDS (red)	IgG as HC + LC	98.5%	98.9%	98.6%	98.5%
CE-SDS (non-red)	Intact IgG	91.3%	90.8%	90.7%	91.2%
icIEF	% acidic isoforms	27.0%	28.8%	34.1%	35.6%
	% main peak	70.3%	68.7%	63.1%	60.5%
	% basic isoforms ¹	< LOQ	< LOQ	< LOQ	< LOQ
SE-HPLC	% monomer	98.6%	98.7%	98.7%	98.5%
	% HMWS	1.4%	1.2%	1.2%	1.3%
	% LMWS ²	< LOQ	0.1%	0.1%	0.2%
Antigen binding	% relative to reference standard	93%	106%	112%	110%
Potency	% relative to reference standard	NT ³	94%	92%	90%
A280	Protein concentration (mg/mL)	20.2 mg/mL	20.5 mg/mL	20.4 mg/mL	20.1 mg/mL
pH	pH	6.0	5.9	6.0	6.0
HC: Heavy chain. HMWS: High molecular weight species. LC: Light chain. LMWS: Low molecular weight species. RS: Reference suspension. BY: Brown-yellow. B: Brown.					
1.	LOQ is 4.9%.				
2.	LOQ is 0.1%.				
3.	No potency testing at TP=0m due to ongoing validation of potency assay.				

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CLAIMS

1. A pharmaceutical composition comprising
 - a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising
 - an antigen-binding region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4, a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7, and
 - an Fc region comprising a mutation in one or more amino acid residues selected from the group corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index;
 - b) 5-40 mM histidine or acetate;
 - c) 100 – 400 mM sorbitol or sucrose; and
 - d) a surfactant.
2. The pharmaceutical composition according to claim 1, consisting essentially of (a), (b), (c) and (d).
3. The pharmaceutical composition according to any one of claims 1 and 2, consisting of (a), (b), (c) and (d) in aqueous solution.
4. The pharmaceutical composition according to any one of claims 1 to 3, wherein the antibody comprises
 - a variable heavy chain (VH) region comprising SEQ ID NO:1 or an amino acid sequence having at least 80% identity, such as 90%, or 95%, or 97%, or 98%, or 99%, to SEQ ID NO:1; and/or
 - a variable light chain (VL) region comprising SEQ ID NO:5 or an amino acid sequence having at least 80% identity, such as 90%, or 95%, or 97%, or 98%, or 99%, to SEQ ID NO:5.
5. The pharmaceutical composition according to claim 4, wherein, in the antibody
 - the VH differs from SEQ ID NO:1 by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues; and/or
 - the VL differs from SEQ ID NO:5 by 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues.

6. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody comprises a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5.
7. The pharmaceutical composition according to any one of the preceding claims, wherein,
5 in the antibody, the mutation in the one or more amino acid residues is selected from the group consisting of E430G, E345K, E430S, E430F, E430T, E345Q, E345R, E345Y, S440Y and S440W, such as from the group corresponding to E430G, E345K, E430S and E345Q.
8. The pharmaceutical composition according to any one of the preceding claims, wherein the mutation in the one or more amino acid residues comprises or consists of E430G.
- 10 9. The pharmaceutical composition according to any one of the preceding claims, wherein the Fc region comprises one or more further mutations in amino acid residues other than those corresponding to E430, E345 and S440 in a human IgG1 heavy chain, and wherein the one or more further mutations do not reduce the complement-dependent cytotoxicity (CDC) and/or antibody-dependent cell-mediated cytotoxicity (ADCC) induced
15 by the antibody without the one or more further mutations.
10. The pharmaceutical composition according to claim 9, wherein the one or more further mutations are 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues.
11. The pharmaceutical composition according to any one of the preceding claims, wherein,
20 in the antibody, the amino acid residue corresponding to Lys (K) at position 447 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index, is absent.
12. The pharmaceutical composition according to any one of the preceding claims, wherein the Fc region is, except for the recited mutations, a human IgG1, IgG2, IgG3 or IgG4
25 isotype or a mixed isotype thereof.
13. The pharmaceutical composition according to any one of the preceding claims, wherein the Fc region is, except for the recited mutations, a human IgG1 Fc region, such as a human IgG1m(f), IgG1m(a), IgG1m(x), IgG1m(z) allotype or a mixed allotype of any two or more thereof.
- 30 14. The pharmaceutical composition according to any one of the preceding claims, wherein the Fc region is a human IgG1 Fc region comprising, except for the recited mutations, amino acid residues 99 to 329 (direct numbering) of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID NO:23 or SEQ ID NO:45.
15. The pharmaceutical composition according to any one of the preceding claims, wherein
35 the Fc region is a human IgG1 Fc region comprising amino acid residues 99 to 329 of

SEQ ID NO:24, SEQ ID NO:25, SEQ ID NO:26, SEQ ID NO:27, SEQ ID NO:28, SEQ ID NO:29, SEQ ID NO:30, SEQ ID NO:31, SEQ ID NO:32, SEQ ID NO:33 or SEQ ID NO:46.

16. A pharmaceutical composition comprising
- a) 1 to 200 mg/mL of an antibody binding to human CD38, comprising
 - 5 - a heavy chain comprising a VH region comprising a VH CDR1 having the sequence as set forth in SEQ ID NO:2, a VH CDR2 having the sequence as set forth in SEQ ID NO:3, a VH CDR3 having the sequence as set forth in SEQ ID NO:4 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, the amino acid residues being numbered according to the EU index, and
 - 10 - a light chain comprising a VL region comprising a VL CDR1 having the sequence as set forth in SEQ ID NO:6, a VL CDR2 having the sequence AAS, and a VL CDR3 having the sequence as set forth in SEQ ID NO:7;
 - b) 5-40 mM histidine or acetate;
 - c) 100 – 400 mM sorbitol or sucrose; and
 - 15 d) a surfactant.
 - 17. A pharmaceutical composition according to claim 16, consisting essentially of (a), (b), (c) and (d).
 - 18. A pharmaceutical composition according to any one of claims 16 and 17, consisting of (a), (b), (c) and (d) in aqueous solution.
 - 20 19. The pharmaceutical composition according to any one of claims 16 to 18, wherein the antibody comprises
 - a heavy chain comprising a VH region comprising SEQ ID NO:1 and a human IgG1 CH region with a mutation in one or more of E430, E345 and S440, wherein the amino acid residue numbering is according to the EU index, and
 - 25 - a light chain comprising a VL comprising SEQ ID NO:5.
 - 20. The pharmaceutical composition according to any one of claims 16 to 19, wherein the mutation comprises or consists of E430G.
 - 21. The pharmaceutical composition according to any one of claims 16 to 20, wherein the human IgG1 CH region is, except for the recited mutations, a human IgG1m(f),
30 IgG1m(a), IgG1m(x) or IgG1m(z) allotype, or a mixed allotype of any two or more thereof.
 - 22. The pharmaceutical composition according to any one of claims 16 to 21, wherein the human IgG1 CH region comprises, except for the recited mutations, the amino acid sequence of SEQ ID NO:19, SEQ ID NO:20, SEQ ID NO:21, SEQ ID NO:22, SEQ ID
35 NO:23 or SEQ ID NO:45.

23. The pharmaceutical composition according to claim 22, wherein the human IgG1 CH region comprises one or more further mutations in amino acid residues other than those corresponding to E430, E345 and S440 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index, optionally wherein said one or more further mutations are 12 or less, such as 11, 10, 9, 8, 7, 6, 5, 4, 3, 2 or 1 mutations such as substitutions, insertions or deletions of amino acid residues.
24. The pharmaceutical composition according to any one of claims 15 to 23, wherein the CH region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:24 to SEQ ID NO:33 and SEQ ID NO:46.
25. The pharmaceutical composition according to claim 24, wherein the CH region comprises SEQ ID NO:24 or SEQ ID NO:46, optionally wherein the light chain comprises a CL comprising SEQ ID NO:37.
26. The pharmaceutical composition according to any one of claims 16 to 25, wherein the amino acid residue corresponding to Lys (K) at position 447 in a human IgG1 heavy chain, wherein the amino acid residues are numbered according to the EU index, is absent.
27. The pharmaceutical composition according to any one of claims 16 to 26, which is a bivalent antibody.
28. The antibody according to any one of the preceding claims, wherein the antibody is, except for the recited mutation(s), a human monoclonal full-length monospecific bivalent IgG1m(f), κ antibody.
29. The pharmaceutical composition according to any one of the preceding claims, wherein a) is 1 to 80 mg/mL, such as 1 to 60 mg/ml, 1 to 40 mg/mL, 1 to 30 mg/ml or 1 to 25 mg/ml; 2 to 80 mg/mL, such as 2 to 40 mg/mL or 2 to 30 mg/ml; or 10 to 80 mg/mL, such as 10 to 40 mg/mL or 10 to 30 mg/ml; or 15 to 80 mg/ml, such as 15 to 40 mg/ml, such as 15 to 25 mg/ml, such as 2 mg/ml, 4 mg/mL, 6 mg/mL, 8 mg/mL, 10 mg/mL, 12 mg/mL, 14 mg/mL, 16 mg/mL, 18 mg/mL, 20 mg/mL, 22 mg/mL, 24 mg/mL, 26 mg/mL, 28 mg/mL, 30 mg/mL, 32 mg/mL, 34 mg/mL, 36 mg/mL, 38 mg/mL, 40 mg/mL, or 50 mg/mL; preferably about 20 mg/mL, such as 20 mg/mL, of the antibody.
30. The pharmaceutical composition according to any one of the preceding claims wherein b) is 5 to 30 mM, such as 5 to 25 mM, such as 10 mM, 11, mM, 12 mM, 13 mM, 14 mM, 15 mM, 16 mM, 17 mM, 18 mM, 19 mM, 20 mM, 21 mM, 22 mM, 23mM, 24 mM, 25 mM, 26 mM, 27 mM, 28 mM, 29 mM or 30 mM; preferably about 20 mM, such as 20 mM of histidine or acetate; such as histidine.
31. The pharmaceutical composition according to any one of the preceding claims, wherein c) is 100 to 350 mM, such as 100 to 300 mM, 100 to 260 mM, 100 to 200 mM, 150 to

- 350 mM, 200 to 300 mM, 200 to 260 mM, 200 to 350 mM, 200 to 300 mM, 200 to 260 mM, 230 to 350 mM, 230 to 300 mM, 230 to 260 mM or 240 to 260 mM; such as 245 mM, 246 mM, 247 mM, 248 mM, 249 mM, 250 mM, 251 mM, 252 mM, 253 mM, 254 mM, or 255 mM, preferably about 250 mM, such as 250 mM of sorbitol or sucrose, such as sorbitol.
32. The pharmaceutical composition according to any one of the preceding claims wherein the pH of the pharmaceutical composition is 5.0 to 6.5, such as 5.5 to 6.5, such as 5.6 to 6.5, 5.7 to 6.5, 5.8 to 6.5, 5.9 to 6.5, 6.0 to 6.5, 5.5 to 6.4, 5.5 to 6.3, 5.5 to 6.2, 5.5 to 6.1, 5.5 to 6.0, 5.7 to 6.3, 5.8 to 6.2, 5.9 to 6.1, such as about 6, such as 6 or 6.0.
33. The pharmaceutical composition according to any one of the preceding claims, wherein the surfactant is selected from the group comprising glycerol monooleate, benzethonium chloride, sodium docusate, phospholipids, polyethylene alkyl ethers, sodium lauryl sulfate and tricaprilyn, benzalkonium chloride, citrimide, cetylpyridinium chloride and phospholipids, alpha tocopherol, glycerol monooleate, myristyl alcohol, phospholipids, poloxamers, polyoxyethylene alkyl ethers, polyoxyethylene castor oil derivatives, polyoxyethylene sorbintan fatty acid esters, polyoxyethylene sterarates, polyoxyl hydroxystearate, polyoxylglycerides, polysorbates, propylene glycol dilaurate, propylene glycol monolaurate, sorbitan esters sucrose palmitate, sucrose stearate, tricaprilyn and TPGS.
34. The pharmaceutical composition according to any one of the preceding claims, wherein the surfactant is a polysorbate.
35. The pharmaceutical composition according to any one of the preceding claims, wherein the surfactant is polysorbate 20 or 80, such as polysorbate 80.
36. The pharmaceutical composition according to any one of the preceding claims, wherein the concentration of the surfactant is from about 0.005% to 0.5% w/v, such as from about 0.01 to 0.1 % w/v, such as from about 0.01 to 0.09 % w/v such as from about 0.01 to 0.06 % w/v such as from about 0.01 to 0.05% w/v such as 0.02% w/v or 0.03% w/v or 0.04% w/v or 0.05% w/v, or 0.06% w/v, such as about 0.04% w/v or 0.04% w/v.
37. The pharmaceutical composition according to any one of the preceding claims, wherein the composition has a pH of 5.9 to 6.1, such as about 6 or 6.0 and comprises or consists essentially of:
- 1 to 80 mg/mL of the antibody
 - 15 to 40 mM histidine
 - 200 to 300 mM sorbitol
 - 0.01% to 0.1 % w/v of a surfactant, preferably polysorbate, such as polysorbate 20 or polysorbate 80, such as polysorbate 80.

38. The pharmaceutical composition according to any one of the preceding claims, wherein the composition has a pH of 5.9 to 6.1, such as about 6 or 6.0 and comprises or consists essentially of:
- a) 10 to 40 mg/mL of the antibody
 - 5 b) 15 to 40 mM histidine
 - c) 200 to 300 mM sorbitol
 - d) 0.02% to 0.06% w/v of a surfactant, preferably polysorbate, such as polysorbate 20 or polysorbate 80, such as polysorbate 80.
39. The pharmaceutical composition according to any one of the preceding claims, wherein the composition has a pH of 5.9 to 6.1, such as about 6 or 6.0 and comprises or consists essentially of:
- a) 10 to 40 mg/mL of the antibody
 - b) 15 to 25 mM histidine
 - c) 240 to 260 mM sorbitol
 - 15 d) 0.02% to 0.06% w/v of a surfactant, preferably polysorbate, such as polysorbate 20 or polysorbate 80, such as polysorbate 80.
40. The pharmaceutical composition according to any one of the preceding claims, wherein the composition is stable for a period of at least 8 weeks, such as for a period of 1-60 months, 1-48 months, 1-36 months, 1-30 months, 1-24 months, 1-18 months, 1-12 months or 1-6 months; such as for 2-60 months, 2-48 months, 2-36 months, 2-30 months, 2-24 months, 2-18 months, 2-12 months or 2-6 months; such as for 3-60 months, 3-48 months, 3-36 months, 3-30 months, 3-24 months, 3-18 months, 3-12 months or 3-6 months, such as for 48 months, 36 months, 30 months, 24 months, 18 months, 12 months, or 6 months.
- 25 41. The pharmaceutical composition according to claim 40, wherein stability is determined when the composition is kept at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$, $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ or $40^{\circ}\text{C} \pm 10^{\circ}\text{C}$, such as at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$, about $25^{\circ}\text{C} \pm 5^{\circ}\text{C}$ or about 40°C , such as at $5^{\circ}\text{C} \pm 3^{\circ}\text{C}$.
42. The pharmaceutical composition according to claim 40 or 41, wherein stability is assessed by
- 30 (a) sub-visible particle count;
 - (b) pH;
 - (c) protein concentration;
 - (d) size-exclusion chromatography;
 - (e) isoelectric focusing;
 - 35 (f) electrophoresis under reducing conditions;

(g) electrophoresis under non-reducing conditions; and/or

(h) visual inspection

43. The pharmaceutical composition according to any one of claims 40 to 42, wherein stability is assessed by determining

5 (a) sub-visible particle count using a micro-flow imaging (MFI) microscope, optionally as provided in Example 12;

(b) pH, optionally as provided in Example 11;

(c) protein concentration using absorbance at 280 nm (A_{280}) optionally as provided in Example 11;

10 (d) relative amount of antibody monomer as compared to the sum of antibody monomer, high molecular weight (HMW) species and low molecular weight (LMW) species present in the composition using size exclusion chromatography, optionally as provided in Example 11;

15 (e) the relative amount of antibody as compared to the sum of antibody, acidic variants of the antibody and basic variants of the antibody by imaged capillary isoelectric focusing (icIEF), optionally as provided in Example 11;

(f) the relative amount of the sum of the light chain and heavy chain as compared to the sum of the light chain, heavy chain and non-glycosylated heavy chain by reduced capillary electrophoresis, optionally as provided by Example 11;

20 (g) the relative amount of antibody peak as compared to the sum of antibody, peaks with molecular weights lower than the antibody peak and peaks with molecular weights higher than the antibody peak; and/or

(h) visual appearance, optionally as provided in Example 11.

25 44. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody is capable of binding to His-tagged human CD38, as specified in SEQ ID NO: 39.

45. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody has an inhibitory effect on the cyclase activity of human CD38.

30 46. The pharmaceutical composition according to claim 45, wherein the antibody inhibits the cyclase activity of human CD38 by at least about 40%, such as at least about 50%, such as at least about 60%, optionally wherein the inhibition of cyclase activity is determined by an assay comprising the steps of:

a) seeding 200,000 Daudi or Wien133 cells in 100 μ L 20 mM Tris-HCL per well; or seeding 0.6 μ g/mL His-tagged soluble human CD38 (SEQ ID NO:39) in 100 μ L 20 mM Tris-HCL per well in a multi-well plate;

- b) adding 1 µg/mL CD38 antibody and 80 µM NGD to each well;
 - c) measuring fluorescence until a plateau is reached (e.g.; 5, 10 or 30 minutes); and
 - d) determining the percentage inhibition as compared to a control, such as a well incubated with an isotype control antibody.
- 5 47. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody induces apoptosis in the presence, but not in the absence, of an Fc-cross-linking antibody.
48. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody induces CDC, ADCC, antibody-dependent cell-phagocytosis (ADCP), trogocytosis,
- 10 or any combination thereof, of cells expressing human CD38.
49. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody induces CDC of cells expressing human CD38.
50. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody induces CDC against Daudi cells (ATCC No. CCL-213) or Ramos cells (ATCC No.
- 15 CRL-1596) resulting in a maximum lysis at least 50%, such at least 60%, such as at least 70% higher than that obtained with a reference antibody differing only in the absence of the one or more mutations in the Fc region.
51. The pharmaceutical composition according to claim 50, wherein the CDC is determined by an assay comprising the steps of:
- 20 a) plating 100,000 CD38-expressing cells in 40 µL culture medium supplemented with 0.2% BSA per well in a multi-well plate;
- b) preincubating cells for 20 minutes with 40 µL of serially diluted CD38 antibody (0.0002-10 µg/mL);
- c) incubating each well for 45 minutes at 37°C with 20 percent of pooled normal human
- 25 serum;
- d) adding a viability dye and measuring the percentage of cell lysis on a flow cytometer;
- e) determining the maximum lysis using non-linear regression.
52. The pharmaceutical composition according to any one of the preceding claims, wherein the antibody is a full-length bivalent antibody comprising, consisting, or consisting essentially
- 30 of two heavy chains and two light chains, wherein
- each heavy chain comprises a VH region and a CH region, wherein the VH region comprises SEQ ID NO:1 and the CH region comprises SEQ ID NO:24 or SEQ ID NO:46, and
 - each light chain comprises a VL region and a CL region, wherein the VL region
- 35 comprises SEQ ID NO:5 and the CL region comprises SEQ ID NO:37.

53. A pharmaceutical composition having a pH of about 6 and comprising or consisting essentially of
- a) about 20 mg/mL of an antibody binding to human CD38,
 - b) about 20 mM histidine,
 - 5 c) about 250 mM sorbitol, and
 - d) about 0.04% w/v of polysorbate 80,
- wherein the antibody is a full-length bivalent antibody comprising, consisting, or consisting essentially of two heavy chains and two light chains, wherein
- each heavy chain comprises a VH region and a CH region, wherein the VH region
10 comprises SEQ ID NO:1 and the CH region comprises SEQ ID NO:24 or SEQ ID NO:46, and
 - each light chain comprises a VL region and a CL region, wherein the VL region comprises SEQ ID NO:5 and the CL region comprises SEQ ID NO:37.
54. The pharmaceutical composition according to claim 53, having a pH of about 6 and
15 comprising, consisting or consisting essentially of
- a) 20 mg/mL of the antibody,
 - b) 20 mM histidine,
 - c) 250 mM sorbitol, and
 - d) 0.04% w/v of polysorbate 80,
- 20 in aqueous solution.
55. The pharmaceutical composition according to any one of claims 52 to 54, wherein the antibody is produced in Chinese hamster ovary (CHO) cells.
56. The pharmaceutical composition according to any one of claims 52 to 55, wherein the antibody is obtained or obtainable by a method comprising expressing the heavy chain and
25 the light chain in a CHO cell.
57. The pharmaceutical composition according to any one of the preceding claims, wherein the composition is for intravenous or subcutaneous injection or infusion.
58. The pharmaceutical composition according to any one of the preceding claims, wherein the composition is a concentrate to be diluted; such as in a 0.9% NaCl (saline) or dextrose
30 solution, optionally a 5% w/v dextrose solution.
59. The pharmaceutical composition according to any one of the preceding claims, for use as a medicament.
60. The pharmaceutical composition according to any one of the preceding claims, for use in treating a disease involving cells expressing CD38.

61. The pharmaceutical composition according to any one of the preceding claims, for use in inducing a CDC-response against a tumor comprising cells expressing CD38.
62. The pharmaceutical composition according to any one of the preceding claims, for use in treating or preventing a cancer in a subject comprising cells expressing human CD38.
- 5 63. The pharmaceutical composition according to any one of the preceding claims, for use in treating a cancer refractory to a prior therapy comprising one or more of a CD38 antibody, a proteasome inhibitor (PI) and an immunomodulatory drug (IMiD).
64. The pharmaceutical composition according to any one of the preceding claims, for use in treating a cancer relapsed after a prior therapy comprising one or more of a CD38 antibody,
10 a PI and an IMiD.
65. The pharmaceutical composition for the use according to any one of claims 63 and 64, wherein the CD38 antibody is daratumumab.
66. The pharmaceutical composition for the use according to any one of claims 62 to 65, wherein the cancer is a hematological cancer.
- 15 67. The pharmaceutical composition for the use according to any one of claims 62 to 65, wherein the cancer is selected from the group consisting of multiple myeloma, chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (adults) (AML), B cell lymphoma, diffuse large B-cell lymphoma (DLBCL) and myelodysplastic syndrome (MDS), optionally wherein the ALL is B cell acute lymphoblastic
20 leukemia (B-ALL).
68. The pharmaceutical composition for the use according to any one of claims 66 and 67, wherein the B cell lymphoma is selected from the group consisting of mantle cell lymphoma, Burkitt's lymphoma and follicular lymphoma (FL).
69. The pharmaceutical composition for the use according to claim 66, wherein the
25 hematological cancer is selected from the group consisting of multiple-myeloma (MM), chronic lymphocytic leukemia (CLL), acute lymphoblastic leukemia (ALL), acute myelogenous leukemia (adults) (AML), mantle cell lymphoma, follicular lymphoma (FL), and diffuse large B-cell lymphoma (DLBCL).
70. The pharmaceutical composition for the use according to any one of claims to 62 to 65,
30 wherein the cancer comprises a solid tumor.
71. The pharmaceutical composition for the use according to claim 70, wherein the solid tumor is melanoma, lung cancer, squamous non-small cell lung cancer (NSCLC), non-squamous NSCLC, colorectal cancer, prostate cancer, castration-resistant prostate cancer, stomach cancer, ovarian cancer, gastric cancer, liver cancer, pancreatic cancer, thyroid cancer,
35 squamous cell carcinoma of the head and neck, carcinoma of the esophagus or

- gastrointestinal tract, breast cancer, fallopian tube cancer, brain cancer, urethral cancer, genitourinary cancer, endometrial cancer, cervical cancer, lung adenocarcinoma, renal cell carcinoma (RCC) (e.g., a kidney clear cell carcinoma or a kidney papillary cell carcinoma), mesothelioma, nasopharyngeal carcinoma (NPC), a carcinoma of the esophagus or
- 5 gastrointestinal tract, or a metastatic lesion of anyone thereof.
72. The antibody for the use according to any one of claims 70 and 71, wherein the solid tumor lacks detectable CD38 expression.
73. The antibody for the use according to any one of claims 62 to 72, wherein the cancer is in a patient comprising T regulatory cells expressing CD38.
- 10 74. The pharmaceutical composition according to any one of claims 1 to 58, for use in treating or preventing rheumatoid arthritis.
75. A method for treating a disease comprising cells expressing CD38, comprising administering the pharmaceutical composition according to any one of claims 1 to 58 to a patient in need thereof.
- 15 76. The method of claim 75, wherein the pharmaceutical composition is administered in a therapeutically effective amount and/or for a time sufficient to treat the disease.
77. The method of any one of claims 75 and 76, further comprising the feature(s) of any one of claims 59 to 73.