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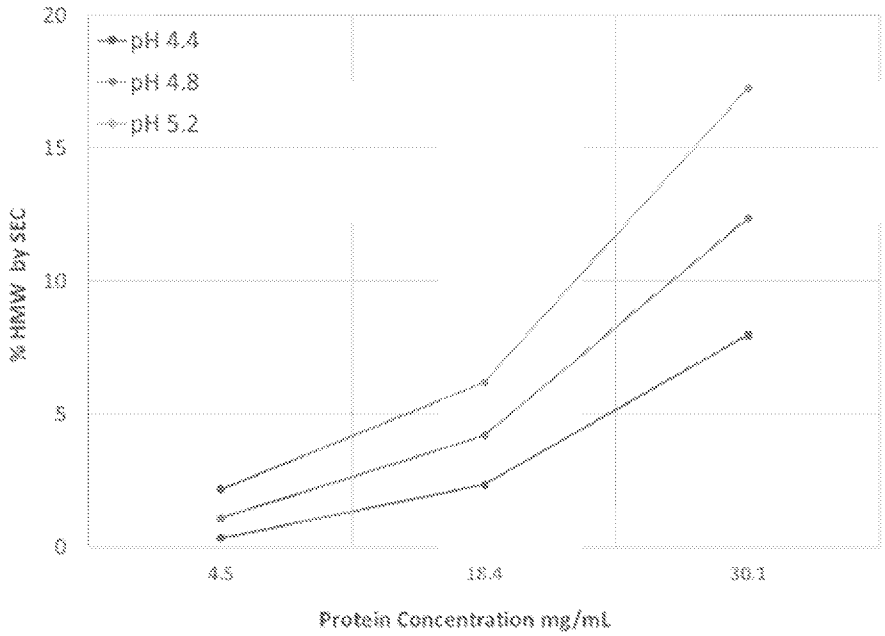
(54) Title: BISPECIFIC MOLECULE STABILIZING COMPOSITION

Fig. 1



(57) Abstract: The present invention provides provide a pharmaceutical composition comprising a bispecific antigen binding molecule at an increased concentration, wherein the composition comprises at least one buffer agent, at least one saccharide; and at least one stabilizing agent selected from Ethylenediaminetetraacetic acid (EDTA), Diethylenetriaminepentetic acid (DTP A), and citric acid in order to stabilize the bispecific antigen-binding agent even at higher concentration.

[Continued on next page]

WO 2024/059675 A2

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BISPECIFIC MOLECULE STABILIZING COMPOSITION

TECHNICAL FIELD

[1] This invention relates to aspects of biotechnology, in particular to the provision of a stabilizing composition for bispecific molecules.

BACKGROUND

[2] Pharmaceuticals which are protein-based, such as recombinant proteins, can be obtained in high purity when first manufactured due to advances in commercial scale purification processes. However, proteins are only marginally stable and are highly susceptible to degradation even during upstream manufacturing, both chemical and physical. Chemical degradation refers to modifications involving covalent bonds, such as deamidation, oxidation, cleavage or formation of new disulfide bridges, hydrolysis, isomerization, or deglycosylation. Physical degradation includes protein unfolding, undesirable adsorption to surfaces, and aggregation. Dealing with these physical and chemical instabilities is one of the most challenging tasks in the development of protein pharmaceuticals (Chi et al., Pharm Res, Vol. 20, No. 9, Sept 2003, pp. 1325-1336, Roberts, Trends Biotechnol. 2014 Jul;32(7):372-80).

[3] Accordingly, despite the advances in manufacturing, new protein-based pharmaceuticals require appropriate formulations in order to avoid product quality impact such as protein aggregation. This affects manufacturing, storage and administration.

[4] Such new protein-based pharmaceuticals comprise, for example, bispecific (monoclonal) antibodies. A bispecific antibody is an artificial protein that can simultaneously bind to two different types of antigens. They are known in several structural formats, and current applications have been explored for cancer immunotherapy and drug delivery (Fan, Gaowei; Wang, Zujian; Hao, Mingju; Li, Jinming (2015). "Bispecific antibodies and their applications". Journal of Hematology & Oncology. 8: 130).

[5] In general, bispecific molecules useful in immunooncology can be antigen-binding polypeptides such as antibodies can be IgG-like, i.e. full length bispecific antibodies, or non-IgG-like bispecific antibodies, which are not full-length antigen-binding molecules. Full length bispecific antibodies typically retain the traditional monoclonal antibody (mAb) structure of two Fab arms and one Fc region, except the two Fab sites bind different antigens. Non full-length bispecific antibodies lack an Fc region entirely. These include chemically linked Fabs, consisting of only the Fab regions, and various types of bivalent and

trivalent single-chain variable fragments (scFvs). There are also fusion proteins mimicking the variable domains of two antibodies. An example of such formats are BiTE® (bi-specific T-cell engagers) molecules (Yang, Fa; Wen, Weihong; Qin, Weijun (2016). "Bispecific Antibodies as a Development Platform for New Concepts and Treatment Strategies". International Journal of Molecular Sciences. 18 (1): 48).

[6] Exemplary bispecific molecules such as BiTE® antigen-binding molecules are recombinant protein constructs made from two flexibly linked antibody derived binding domains. One binding domain of BiTE® antigen-binding molecules is specific for a selected tumor-associated surface antigen on target cells; the second binding domain is specific for CD3, a subunit of the T cell receptor complex on T cells. By their particular design BiTE® antigen-binding molecules are uniquely suited to transiently connect T cells with target cells and, at the same time, potently activate the inherent cytolytic potential of T cells against target cells. An important further development of the first generation of BiTE® antigen-binding molecules (see WO 99/54440 and WO 2005/040220) developed into the clinic as AMG 103 and AMG 110 was the provision of bispecific antigen-binding molecules binding to a context independent epitope at the N-terminus of the CD3ε chain (WO 2008/119567). BiTE® antigen-binding molecules binding to this elected epitope do not only show cross-species specificity for human and *Callithrix jacchus, Saguinus oedipus* or *Saimiri sciureus* CD3ε chain, but also, due to recognizing this specific epitope instead of previously described epitopes for CD3 binders in bispecific T cell engaging molecules, do not unspecifically activate T cells to the same degree as observed for the previous generation of T cell engaging antibodies. This reduction in T cell activation was connected with less or reduced T cell redistribution in patients, which was identified as a risk for side effects.

[7] Currently, about 1 to 5 mg/mL (i.e. 0.1 to 0.5% (w/v)) is a typical concentration of bispecific antigen-binding molecules such as BiTE® (bispecific T-cell engager) molecules conjugated to a single-chain Fc (scFc BiTE® molecule) in liquid (reconstituted) pharmaceutical compositions in order to avoid aggregation such as HMW (high molecular weight) species formation. Such liquid compositions are typically lyophilized for storage and reconstituted for patient administration, typically by infusion. However, higher concentrations of bispecific antigen-binding molecules would be desirable in order to meet the required dosage within a limited volume, e.g. for subcutaneous administration. Further, higher bispecific antigen-binding molecule concentrations means reduced buffer volume, less resources and less energy consumption, e.g. for development and manufacturing. Hence, there is a need to provide means to reduce the risk of aggregation in order to obtain stable high concentration formulations of bispecific antigen-binding molecules.

SUMMARY

[8] Surprisingly, a liquid pharmaceutical composition comprising a stabilizing agent selected from Ethylenediaminetetraacetic acid (EDTA), Diethylenetriaminepentetic acid (DTPA), and citric acid, wherein the stabilizing agent is present preferably in a concentration in the range of 0.005% to 0.25% (w/v), preferably 0.01 to 0.2% (w/v), ensures improved bispecific antigen-binding molecule product

quality at higher concentrations of about 8 to 35 mg/ml, hence facilitating (i.) higher dosage within a limited volume leading to drug applications which require stable drug at high concentration such as subcutaneous administration to a patient in need thereof, and (ii.) less resources consuming production and storage of liquid, lyophilized and reconstituted pharmaceutical compositions comprising bispecific antigen-binding molecules such as scFc BiTE® molecules.

[9] Hence, in one aspect, it is envisaged in the context of the present invention to provide a

liquid pharmaceutical composition comprising

(a) a bispecific antigen-binding molecule comprising at least three domains, wherein:

- a first domain binds to a target cell surface antigen, wherein the target cell surface antigen is a tumor antigen;
- a second domain binds to an extracellular epitope of the human and/or the Macaca CD3 chain; and
- a third domain comprises two polypeptide monomers, each comprising a hinge, a CH2 domain and a CH3 domain, wherein said two polypeptide monomers are fused to each other via a peptide linker, and wherein said third domain comprises in an amino to carboxyl order: hinge-CH2-CH3-linker-hinge-CH2-CH3;

wherein the concentration of the bispecific antigen-binding molecule is 8 to 35 mg/ml;

(b) at least one buffer agent;

(c) at least one saccharide; and

(d) at least one stabilizing agent selected from Ethylenediaminetetraacetic acid (EDTA), Diethylenetriaminepentetic acid (DTPA), and citric acid, wherein the stabilizing agent is present in a concentration in the range of 0.005% to 0.25% (w/v), preferably 0.01 to 0.2% (w/v);

and wherein the pH of the pharmaceutical composition is in the range of 4.0 to 6.0.

[10] According to said aspect, it is also envisaged that the bispecific antigen-binding molecule is a single chain molecule.

[11] According to said aspect, it is also envisaged that the bispecific antigen-binding molecule is half-life extended.

[12] According to said aspect, it is also envisaged that a glycosylation site at Kabat position 314 of the CH2 domains in the third domain of the bispecific antigen-binding molecule is removed by a N314X substitution, wherein X is any amino acid excluding Q.

[13] According to said aspect, it is also envisaged that the each of said polypeptide monomers of the third domain has an amino acid sequence that is at least 90% identical to a sequence selected from the

group consisting of: SEQ ID NOs: 17-24, or has an amino acid sequence selected from the group consisting of SEQ ID NOs: 17-24.

[14] According to said aspect, it is also envisaged that the CH2 domain comprises an intra domain cysteine disulfide bridge.

[15] According to said aspect, it is also envisaged that the tumor antigen is selected from the group consisting of CDH19, CDH3, MSLN, DLL3, FLT3, EGFRvIII, BCMA, PSMA, CD33, CD19, CD20, CLDN18.2, MUC17, EpCAM, CD70, and CLDN6.

[16] According to said aspect, it is also envisaged that second domain is an extracellular epitope of the human and/or the Macaca CD3ε chain.

[17] According to said aspect, it is also envisaged that

(i) the first domain comprises two antibody variable domains and the second domain comprises two antibody variable domains;
(ii) the first domain comprises one antibody variable domain and the second domain comprises two antibody variable domains;
(iii) the first domain comprises two antibody variable domains and the second domain comprises one antibody variable domain; or
(iv) the first domain comprises one antibody variable domain and the second domain comprises one antibody variable domain.

[18] According to said aspect, it is also envisaged that the antibody construct comprises in an amino to carboxyl order:

(a) the first domain;
(b) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 187-189;
(c) the second domain;
(d) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 187, 188, 189, 195, 196, 197 and 198;
(e) the first polypeptide monomer of the third domain;
(f) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 191, 192, 193 and 194; and
(g) the second polypeptide monomer of the third domain.

[19] According to said aspect, it is also envisaged that the first binding domain of the construct comprises a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of:

(a) CDR-H1 as depicted in SEQ ID NO: 4, CDR-H2 as depicted in SEQ ID NO: 5, CDR-H3 as depicted in SEQ ID NO: 6, CDR-L1 as depicted in SEQ ID NO: 1, CDR-L2 as depicted in SEQ ID NO: 2 and CDR-L3 as depicted in SEQ ID NO: 3,

(b) CDR-H1 as depicted in SEQ ID NO: 29, CDR-H2 as depicted in SEQ ID NO: 30, CDR-H3 as depicted in SEQ ID NO: 31, CDR-L1 as depicted in SEQ ID NO: 34, CDR-L2 as depicted in SEQ ID NO: 35 and CDR-L3 as depicted in SEQ ID NO: 36,

(c) CDR-H1 as depicted in SEQ ID NO: 42, CDR-H2 as depicted in SEQ ID NO: 43, CDR-H3 as depicted in SEQ ID NO: 44, CDR-L1 as depicted in SEQ ID NO: 45, CDR-L2 as depicted in SEQ ID NO: 46 and CDR-L3 as depicted in SEQ ID NO: 47,

(d) CDR-H1 as depicted in SEQ ID NO: 53, CDR-H2 as depicted in SEQ ID NO: 54, CDR-H3 as depicted in SEQ ID NO: 55, CDR-L1 as depicted in SEQ ID NO: 56, CDR-L2 as depicted in SEQ ID NO: 57 and CDR-L3 as depicted in SEQ ID NO: 58,

(e) CDR-H1 as depicted in SEQ ID NO: 65, CDR-H2 as depicted in SEQ ID NO: 66, CDR-H3 as depicted in SEQ ID NO: 67, CDR-L1 as depicted in SEQ ID NO: 68, CDR-L2 as depicted in SEQ ID NO: 69 and CDR-L3 as depicted in SEQ ID NO: 70,

(f) CDR-H1 as depicted in SEQ ID NO: 83, CDR-H2 as depicted in SEQ ID NO: 84, CDR-H3 as depicted in SEQ ID NO: 85, CDR-L1 as depicted in SEQ ID NO: 86, CDR-L2 as depicted in SEQ ID NO: 87 and CDR-L3 as depicted in SEQ ID NO: 88,

(g) CDR-H1 as depicted in SEQ ID NO: 94, CDR-H2 as depicted in SEQ ID NO: 95, CDR-H3 as depicted in SEQ ID NO: 96, CDR-L1 as depicted in SEQ ID NO: 97, CDR-L2 as depicted in SEQ ID NO: 98 and CDR-L3 as depicted in SEQ ID NO: 99,

(h) CDR-H1 as depicted in SEQ ID NO: 105, CDR-H2 as depicted in SEQ ID NO: 106, CDR-H3 as depicted in SEQ ID NO: 107, CDR-L1 as depicted in SEQ ID NO: 109, CDR-L2 as depicted in SEQ ID NO: 110 and CDR-L3 as depicted in SEQ ID NO: 111,

(i) CDR-H1 as depicted in SEQ ID NO: 115, CDR-H2 as depicted in SEQ ID NO: 116, CDR-H3 as depicted in SEQ ID NO: 117, CDR-L1 as depicted in SEQ ID NO: 118, CDR-L2 as depicted in SEQ ID NO: 119 and CDR-L3 as depicted in SEQ ID NO: 120,

(j) CDR-H1 as depicted in SEQ ID NO: 126, CDR-H2 as depicted in SEQ ID NO: 127, CDR-H3 as depicted in SEQ ID NO: 128, CDR-L1 as depicted in SEQ ID NO: 129, CDR-L2 as depicted in SEQ ID NO: 130 and CDR-L3 as depicted in SEQ ID NO: 131,

(k) CDR-H1 as depicted in SEQ ID NO: 137, CDR-H2 as depicted in SEQ ID NO: 138, CDR-H3 as depicted in SEQ ID NO: 139, CDR-L1 as depicted in SEQ ID NO: 140, CDR-L2 as depicted in SEQ ID NO: 141 and CDR-L3 as depicted in SEQ ID NO: 142,

(l) CDR-H1 as depicted in SEQ ID NO: 152, CDR-H2 as depicted in SEQ ID NO: 153, CDR-H3 as depicted in SEQ ID NO: 154, CDR-L1 as depicted in SEQ ID NO: 155, CDR-L2 as depicted in SEQ ID NO: 156 and CDR-L3 as depicted in SEQ ID NO: 157,

(m) CDR-H1 as depicted in SEQ ID NO: 167, CDR-H2 as depicted in SEQ ID NO: 168, CDR-H3 as depicted in SEQ ID NO: 169, CDR-L1 as depicted in SEQ ID NO: 170, CDR-L2 as depicted in SEQ ID NO: 171 and CDR-L3 as depicted in SEQ ID NO: 172,

(n) CDR-H1 as depicted in SEQ ID NO: 203, CDR-H2 as depicted in SEQ ID NO: 204, CDR-H3 as depicted in SEQ ID NO: 205, CDR-L1 as depicted in SEQ ID NO: 206, CDR-L2 as depicted in SEQ ID NO: 207 and CDR-L3 as depicted in SEQ ID NO: 208;

(o) CDR-H1 as depicted in SEQ ID NO: 214, CDR-H2 as depicted in SEQ ID NO: 215, CDR-H3 as depicted in SEQ ID NO: 216, CDR-L1 as depicted in SEQ ID NO: 217, CDR-L2 as depicted in SEQ ID NO: 218 and CDR-L3 as depicted in SEQ ID NO: 219;

(p) CDR-H1 as depicted in SEQ ID NO: 226, CDR-H2 as depicted in SEQ ID NO: 227, CDR-H3 as depicted in SEQ ID NO: 228, CDR-L1 as depicted in SEQ ID NO: 229, CDR-L2 as depicted in SEQ ID NO: 230 and CDR-L3 as depicted in SEQ ID NO: 231;

(q) CDR-H1 as depicted in SEQ ID NO: 238, CDR-H2 as depicted in SEQ ID NO: 239, CDR-H3 as depicted in SEQ ID NO: 240, CDR-L1 as depicted in SEQ ID NO: 241, CDR-L2 as depicted in SEQ ID NO: 242 and CDR-L3 as depicted in SEQ ID NO: 243; and

(r) CDR-H1 as depicted in SEQ ID NO: 248, CDR-H2 as depicted in SEQ ID NO: 249, CDR-H3 as depicted in SEQ ID NO: 250, CDR-L1 as depicted in SEQ ID NO: 251, CDR-L2 as depicted in SEQ ID NO: 252 and CDR-L3 as depicted in SEQ ID NO: 253.

[20] According to said aspect, it is also envisaged that the concentration of the bispecific antigen-binding molecule is 10 to 35 or 15 to 31 mg/ml, more preferably 20 to 30 mg/ml or 25 to 30 mg/ml.

[21] According to said aspect, it is also envisaged that the concentration of EDTA is in the range of 0.01% to 0.2% (w/V), preferably in the range of 0.01% to 0.16% (w/V), more preferably 0.04% (w/V).

[22] According to said aspect, it is also envisaged that the at least one buffer agent is an acid selected from the group consisting of acetate, glutamate, citrate, succinate, tartrate, fumarate, maleate, histidine, phosphate, 2-(N-morpholino)ethanesulfonate or a combination thereof, preferably glutamate.

[23] According to said aspect, it is also envisaged that the at least one buffer agent is present at a concentration range of 5 to 200 mM, more preferably at a concentration range of 10 to 50 mM, preferably 15 mM.

[24] According to said aspect, it is also envisaged that the at least one saccharide is selected from the group consisting of monosaccharide, disaccharide, cyclic polysaccharide, sugar alcohol, linear branched dextran or linear non-branched dextran.

[25] According to said aspect, it is also envisaged that the disaccharide is selected from the group consisting of sucrose and trehalose and a combination thereof, preferably sucrose.

[26] According to said aspect, it is also envisaged that the sugar alcohol is selected from the group consisting of mannitol and sorbitol and combination thereof.

[27] According to said aspect, it is also envisaged that the at least one saccharide is present at a concentration in the range of 1 to 15% (w/V), preferably in a concentration range of 8 to 12% (w/V), such as 8% (w/V).

[28] According to said aspect, it is also envisaged that further comprising at least one surfactant is selected from the group consisting of polysorbate 20, polysorbate 40, polysorbate 60, polysorbate 80, poloxamer 188, pluronic F68, triton X-100, polyoxyethylen, PEG 3350, PEG 4000 and combinations thereof.

[29] According to said aspect, it is also envisaged that the composition comprises at least one surfactant at a concentration in the range of 0.004 to 0.5% (w/V), preferably in the range of 0.01 to 0.1% (w/V).

[30] According to said aspect, it is also envisaged that the pH of the composition is in the range of 4.0 to 5.3, preferably 4.2 to 5.2, more preferably 4.3 to 4.6.

[31] According to said aspect, it is also envisaged that the pharmaceutical composition has an osmolarity is in the range of 150 to 500 mOsm.

[32] According to said aspect, it is also envisaged that the pharmaceutical composition further comprises an excipient selected from the group consisting of one or more polyol, preferably hydroxypropyl-ß-cyclodextrin, and one or more amino acid, preferably phenylalanine, but preferably not arginine, proline, and tryptophane.

[33] According to said aspect, it is also envisaged that said one or more excipient is present in the concentration range of 0.1 to 15 % (w/V).

[34] According to said aspect, it is also envisaged that the composition comprises

(a) the bispecific antigen-binding molecule according to the invention in a concentration in the range of 8 to 35 mg/ml, preferably 15 to 30 mg/ml,
(b) 15 mM glutamate or acetate,
(c) 8% (w/V) sucrose or 8% (w/V) sucrose and 1% (w/V) hydroxypropyl-β-cyclodextrin,
(d) optionally 0.01% (w/V) polysorbate 80

and wherein the pH of the liquid pharmaceutical composition is any value in the range of 4.0 to 5.2, preferably 4.2 to 4.6 or 4.3 to 4.6.

[35] According to another aspect, it is also envisaged that a solid pharmaceutical composition is obtainable by lyophilization of the liquid pharmaceutical composition of the present invention.

[36] According to another aspect, it is also envisaged that a liquid pharmaceutical composition is obtainable by reconstituting the solid pharmaceutical composition according to the present invention with a pharmaceutically acceptable liquid.

[37] According to another aspect, it is also envisaged that the pharmaceutical composition of the present invention is for use in the treatment of a disease, preferably of a proliferative disease such as cancer, preferably by intravenous or subcutaneous administration.

[38] According to another aspect, it is also envisaged that the liquid pharmaceutical composition of the present invention is used for reducing the formation of high molecular weight species (HMWS) during storage, wherein the amount of HMWS is kept below 5%, preferably below 3% or 2%, if the liquid pharmaceutical composition is stored at or below 4°C, preferably at or below -30°C.

DESCRIPTION OF THE FIGURES

[39] **Figure 1**: FIG. 1 shows percental high molecular weight (HMW) species as determined by size exclusion chromatography (SEC) analysis depending on protein concentration (4.5, 18.4 and 30.1 mg/ml of DLL3xCD3 bispecific antigen-binding molecule) and pH value (pH 4.4 (lower graph), pH 4.8 (middle graph) and pH 5.2 (upper graph)). The liquid pharmaceutical composition comprises 15 mmol glutamic acid, 8% (w/v) sucrose and 0.01% (w/v) polysorbate 80 but no additional stabilizing agent such as EDTA. Percental HMW species increase significantly with increasing pH and protein concentration.

[40] **Figure 2**: FIG. 2 shows percental high molecular weight (HMW) species and low molecular weight (LMW) species after 4 weeks storage at 40°C as determined by size exclusion chromatography (SEC) analysis depending on pH value (individual values in the range of pH 4.1 to 4.9). The liquid pharmaceutical

composition comprises 20 mg/ml of respective bispecific antigen-binding molecule, 15 mmol glutamic acid, 8% (w/v) sucrose and 0.01% (w/v) polysorbate 80 but no additional stabilizing agent such as EDTA. Open symbol stands for HMW and closed symbol for LMW data point. Squares stand for BCMAxCD3 bispecific antigen-binding molecule, circles for CD33xCD3 bispecific antigen-binding molecule and triangles for DLL3xCD3 bispecific antigen-binding molecule. Percental HMW species increase significantly with increasing pH value and percental LMW species decrease with increasing pH value.

[41] **Figure 3**: FIG. 3 shows percental low molecular weight (LMW) species at before storage (t=0, open circles) and after 4 weeks storage at 40°C (close circles) as determined by size exclusion chromatography (SEC) analysis depending on pH value (individual values in the range of pH 4.1 to 4.9). The liquid pharmaceutical composition comprises 5 mg/ml (dark symbols) or 20 mg/ml (light symbols) of respective bispecific antigen-binding molecule. Each formulation comprises 15 mmol glutamic acid and 0.01% (w/v) polysorbate 80, and in addition (from left column to right column): 5% (w/v) sucrose, 5% plus 1% (w/v) Hydroxypropyl-ß-cyclodextrin (HpbCD), sucrose, 5% (w/v) plus 10 mM phenylalanine (Phe), and 0.3 M proline (Pro). The top row "1" depicts results for DLL3xCD3 bispecific antigen-binding molecule, the middle row "2" depicts results for BCMAxCD3 bispecific antigen-binding molecule, and the bottom row "3" depicts results for CD33xCD3 bispecific antigen-binding molecule.

[42] **Figure 4**: **FIG. 4A** shows percental high molecular weight (HMW) species of BCMAxCD3 bispecific antigen-binding molecule from left to right in each set of four columns: (i. ., black) before storage at t=0, (ii., medium grey) after 2 weeks storage at -30°C, (iii., light grey) after 4 weeks storage at 4°C and (iv., dark grey) after 4 weeks storage at 40°C, respectively, as determined by size exclusion chromatography (SEC) analysis depending on pH value (for each set of excipients from left to right pH 4.3 ("4" in x-axis caption), 4.6 ("46" in x-axis caption) and 5.2 ("5" in x-axis caption). The liquid pharmaceutical composition comprises 30 mg/ml of BCMAxCD3 bispecific antigen-binding molecule, 15 mmol glutamic acid, 8% (w/v) sucrose and 0.01% (w/v) polysorbate 80, and from left to right for each triplet of four columns (a) no additional stabilizing agent (control), (b., "CD") 1% (w/v) Hydroxypropyl-ß-cyclodextrin (HpbCD), (c., "Phe") 50 mM phenylalanine, (d., "EDTA") 0.04% (w/v) EDTA, (e., "R") 50 mM arginine, (f., "Trp") 20 mM tryptophane, (g., "Pro") 50 mM proline, and (h., "BA") 0.45% (w/v) benzyl alcohol. **FIG 4B** shows percental high molecular weight (HMW) species of DLL3xCD3 bispecific antigen-binding molecule from left to right in each set of four columns: (i., black) before storage at t=0, (ii., medium grey) after 2 weeks storage at -30°C, (iii., light grey) after 4 weeks storage at 4°C and (iv., dark grey) after 4 weeks storage at 40°C, respectively, as determined by size exclusion chromatography (SEC) analysis depending on pH value (for each set of excipients from left to right pH 4.3 ("4" in x-axis caption), 4.6 ("46" in x-axis caption) and 5.2 ("5" in x-axis caption). The liquid pharmaceutical composition comprises 30 mg/ml of DLL3xCD3 bispecific antigen-binding molecule, 15 mmol glutamic acid, 8% (w/v) sucrose and 0.01% (w/v) polysorbate 80, and from left to right for each triplet of four columns (a.) no additional stabilizing agent (control), (b., "CD") 1% (w/v) Hydroxypropyl-ß-cyclodextrin

(HpbCD), (c., "Phe") 50 mM phenylalanine, (d., "EDTA") 0.04% (w/v) EDTA, (e., "R") 50 mM arginine, (f., "Trp") 20 mM tryptophane, (g., "Pro") 50 mM proline, and (h., "BA") 0.45% (w/v) benzyl alcohol.

[43] **Figure 5**: FIG. 5 shows percental high molecular weight (HMW) species of MUC17xCD3 bispecific antigen-binding molecule from left to right in each set of four columns: (i., black) before storage at t=0, (ii., medium grey) after 4 weeks storage at -30°C, (iii., light grey) after 4 weeks storage at 4°C and (iv., dark grey) after 4 weeks storage at 40°C, respectively, as determined by size exclusion chromatography (SEC) analysis depending on pH value (for each set of excipients from left to right pH 4.2 ("4" in x-axis caption), 4.6 ("46" in x-axis caption) and 5.0 ("5" in x-axis caption). The liquid pharmaceutical composition comprises 16 mg/ml of MUC17xCD3 bispecific antigen-binding molecule, 15 mmol glutamic acid, 8% (w/v) sucrose and 0.01% (w/v) polysorbate 80, and from left to right for each triplet of four columns (a.) no additional stabilizing agent (control), (b., "CD") 1% (w/v) Hydroxypropyl-ß-cyclodextrin (HpbCD), (c., "Phe") 50 mM phenylalanine, (d., "EDTAAp01") 0.01% (w/v) EDTA, (e., "R") 50 mM arginine HCL, (vi., "EDTAAp04") 0.04% (w/v) EDTA, (g., "Pro") 50 M proline, and (h., "EDTAAp16") 0.16% (w/v) EDTA.

DETAILED DESCRIPTION

[44] To increase convenience of delivery of bispecific antigen-binding molecules such as half-life extended (HLE) scFc BiTE® molecules, the provision of a subcutaneous liquid formulation is strongly desired. However, high concentration formulations are often needed to meet the required dosage within a limited volume. Unlike typical concentrations of mAbs of up to 70 mg/ml, bispecific antigen-binding molecules are typically lyophilized with a final reconstitution concentration of about 1 to 5 mg/ml (0.1 to 0.5% (w/v)). In addition, artificial bispecific antigen-binding molecules are more prone to aggregation during product manufacture, storage as well as in-use administration compared to mAbs. It is challenging to formulate bispecific antigen-binding molecules in liquid pharmaceutical compositions at a concentration above about 5 mg/ml(w/v) with minimized undesired aggregation.

[45] Hence, it was surprising that bispecific antigen-binding molecules such as HLE BiTE® molecules at concentrations significantly above 5 mg/ml and up to about 35 mg/ml were stabilized, i.e. were found to show significantly lower %HMW in optimized formulations relative to formulation controls, at the same tested conditions with respect to pH, incubation temperature and period. For example, for DLL3xCD3, MUC17xCD3 and BCMAxCD3 bispecific antigen-binding molecules, respectively, a stabilizing agent with chelating properties such as EDTA (Ethylenediaminetetraacetic acid) significantly reduced %HMW as compared to control formulations over a 4 week period. For illustration, at time t=0, BCMAxCD3 bispecific antigen-binding molecules at 27 mg/m and pH 5.2 showed about 2% HMW with EDTA as stabilizing agent vs. about 12% in a pharmaceutical composition without EDTA, ETPA or citric acid or the like. Other tested excipients like hydroxypropyl-ß-cyclodextrin (HpbCD) and amino acid phenylalanine (Phe) also showed some mitigation of %HMW at certain conditions, especially at a pH close to 4 rather than 5, but less than stabilizing agents with chelating properties. On the other hand, Arg HCl

promoted aggregation by increasing %HMW. Other tested excipients such as Trp and Pro, appeared not to have an effect on %HMW compared to control buffer without a (potentially) stabilizing agent.

[46] The present invention shows for the first time that providing a formulation optimized just by the addition of one selected stabilizing agent can mitigate %HMW for various bispecific antigen-binding molecules at such high concentration in liquid format and that they can be employed for additional administration purposes and may overcome concentration related stability disadvantages of bispecific antigen-binding molecules in comparison to conventional mAbs.

[47] In the context of the present invention, it has been surprisingly achieved to stabilize bispecific antigen-binding molecules at concentrations higher than 5 mg/ml as typically achieved for such molecules, e.g. BiTE molecules, i.e. 8 mg/ml and more, e.g. 8, 9, 10, 11, 12, 13, 14 mg/ml, preferably even at 15 to 35 mg/ml, i.e. 15, 16, 17, 18, 19, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34 or 35 mg/ml by means of the addition of a stabilizing agent, preferably EDTA, DFPA or citric acid. This is surprising as chelating agents such as EDTA have -in addition to the capacity as a chelator- previously been employed to reduce oxidation stress in mAbs. While oxidation as a chemical modification of a molecule in question may contribute to changes of said molecule, protein aggregation is a complicated phenomenon which may go through different pathways with various mechanisms. Bispecific antigen-binding molecules of the present invention, when not formulated in a pharmaceutical composition comprising a stabilizing agent as described herein, do show increasing %HMWS over time as determined by SE-UHPLC (see Table 1). However, chemical modification in terms of deamidation (e.g. N350 and N353) or oxidation (e.g. at methionine position M281) were not observed in bispecific antigen-binding molecules according to the present invention. Without wanting to be bound by theory, the underlying principle of stabilization resides in the interaction of the stabilizing agent with chelating properties with the CD3 binding domain. As determined by differential scanning fluorimetry (DSF) measuring, i.e. a typically rapid screening method to identify low-molecular-weight ligands that bind and stabilize purified protein, the melding temperature Tm of the bispecific antigen-binding molecules of the invention typically rise in presence of the stabilizing agent such as EDTA indicating increasing conformation stability of the CD3 binding domain. Accordingly, molecules of the invention such as SEQ ID NO: 104 and SEQ ID NO: 258 which have even different CD3 binding domains abbreviated as I2C vs. I2E in the sequence table (table 5), respectively, are both stabilized by stabilizing agents with chelating properties of the invention, such as EDTA.

Table 1: Overview of exemplary bispecific antigen-binding molecules %HMW formation over time in the absence of chemical modification occurrence over time.

Test Method	Attributes	T=0	1 MO	3 MO	6 MO	9 MO	12 MO	18 MO	24 MO
Color	N/A	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless	Colorless
Clarity	N/A	< Ref I	< Ref I	< Ref I	< Ref I	< Ref I	< Ref I	< Ref I	< Ref I
pH	N/A	4.4	4.2	4.3	4.3	4.3	4.2	4.3	4.2
SE-UHPLC	% HMWS	0.50%	1.00%	2.20%	4.10%	5.00%	5.00%	7.10%	8.30%
Multi-attribute method (MAM)	% Isomerization (D31)	< 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%	< 0.05%
	% Oxidation (W104)	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%	< 1.0%
	% Deamidation (N330+N384) S3)	< 1.7%	< 1.7%	< 1.7%	< 1.7%	< 1.7%	< 1.7%	< 1.7%	< 1.7%
	% Oxidation (M281)	0.10%	< 0.1%	0.10%	0.10%	< 0.1%	0.10%	0.20%	0.10%
	% Ozone Oxidation (W180)	< 0.03%	0.03%	0.03%	< 0.03%	< 0.03%	0.10%	0.10%	0.10%
	% Oxidation (W385)	0.10%	0.10%	0.10%	0.10%	0.10%	0.10%	0.10%	0.10%

[48] In the context of the present invention, HMWS are primarily dimers, which share similar conformation of monomer, and may be regarded as native-state dimers. HMWS as understood in the context of the present invention are highly reversible, i.e. dimer will dissociate to monomer after 24 hours post thaw hold at 25 °C for multiple bispecific antigen-binding molecules. As shown in exemplary table 1, HMWS increased WITHOUT correspondingly increase in chemical modifications, either Met oxidization or deamidation.

[49] Advantageously, the pharmaceutical composition of the present invention is capable to stabilize bispecific antigen-binding molecules such as HLE BiTE molecules at higher concentrations even at higher pH values which may beneficial for specific administration settings where a too low pH of the pharmaceutical composition is not feasible or where stability of a particular bispecific antigen-binding molecule requires a slightly higher pH value than about 4, e.g. 4.6 or 5.2.

[50] Bispecific antigen-binding molecule concentration may be in the range from 8 to 35 mg/ml, preferably 15 to 30 mg/ml. The stabilizing agent such as EDTA may be at a concentration in the range of about 0.005 to 0.25% (w/v), wherein the upper limit is considered safe for human administration according to applicable guidelines. EDTA may be present as a salt, e.g. disodium EDTA, sodium calcium edetate, and tetrasodium EDTA, preferably disodium EDTA.

[51] Bispecific antigen-binding molecules according to the present invention such as ScFc-BiTE® molecules typically contain a Fc region which are similar in size, pI and hydrophobicity to corresponding regions of IgG antibodies. The general downstream processing of IgG antibodies from host cell proteins (HCPs), other bioreactor impurities and reagents, high molecular weight (HMW) species or aggregates,

low molecular weight species or clips is known in the art (Shukla et al. 2006). Such downstream processing has been adapted for the purification of bispecific antigen-binding molecules such as scFc-BITE® typically comprising the following steps: cell culture harvest; Protein A chromatography; filter virus inactivation; second-column chromatographic polishing step or two, for example CEX; viral filtration; UF/DF

[52] The term "antibody product" refers to "secreted protein" or "secreted recombinant protein" and means a protein (e.g., a recombinant protein) that originally contained at least one secretion signal sequence when it is translated within a mammalian cell, and through, at least in part, enzymatic cleavage of the secretion signal sequence in the mammalian cell, is secreted at least partially into the extracellular space (e.g., a liquid culture medium). Skilled practitioners will appreciate that a "secreted" protein need not dissociate entirely from the cell to be considered a secreted protein.

[53] The term bispecific antibody product encompasses bispecific antibodies such as full length e.g. IgG-based antibodies as well as fragments therefor, which are typically referred to herein as bispecific antigen-binding molecules.

[54] The term "antigen-binding molecule" refers to a molecule in which the structure and/or function is/are based on the structure and/or function of an antibody, *e.g.*, of a full-length or whole immunoglobulin molecule and/or is/are drawn from the variable heavy chain (VH) and/or variable light chain (VL) domains of an antibody or fragment thereof. An antigen-binding molecule is hence capable of binding to its specific target or antigen. Furthermore, the binding domain of an antigen-binding molecule according to the invention comprises the minimum structural requirements of an antibody which allow for the target binding. This minimum requirement may *e.g.* be defined by the presence of at least the three light chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VL region) and/or the three heavy chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VH region), preferably of all six CDRs. An alternative approach to define the minimal structure requirements of an antibody is the definition of the epitope of the antibody within the structure of the specific target, respectively, the protein domain of the target protein composing the epitope region (epitope cluster) or by reference to an specific antibody competing with the epitope of the defined antibody. The antibodies on which the constructs according to the invention are based include for example monoclonal, recombinant, chimeric, deimmunized, humanized and human antibodies.

[55] The binding domain of an antigen-binding molecule according to the invention may e.g. comprise the above referred groups of CDRs. Preferably, those CDRs are comprised in the framework of an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH); however, it does not have to comprise both. Fd fragments, for example, have two VH regions and often retain some antigen-binding function of the intact antigen-binding domain. Additional examples for the format of antibody fragments, antibody variants or binding domains include (1) a Fab fragment, a monovalent fragment having the VL, VH, CL and CH1 domains; (2) a $F(ab')_2$ fragment, a bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; (3) an Fd fragment having the two VH and CH1 domains;

(4) an Fv fragment having the VL and VH domains of a single arm of an antibody, (5) a dAb fragment (Ward et al., (1989) Nature 341 :544-546), which has a VH domain; (6) an isolated complementarity determining region (CDR), and (7) a single chain Fv (scFv) , the latter being preferred (for example, derived from an scFV-library). Examples for embodiments of antigen-binding molecules according to the invention are e.g. described in WO 00/006605, WO 2005/040220, WO 2008/119567, WO 2010/037838, WO 2013/026837, WO 2013/026833, US 2014/0308285, US 2014/0302037, WO 2014/144722, WO 2014/151910, and WO 2015/048272.

[56] Also within the definition of "binding domain" or "domain which binds" are fragments of full-length antibodies, such as VH, VHH, VL, (s)dAb, Fv, Fd, Fab, Fab', F(ab')2 or "r IgG" ("half antibody"). Antigen-binding molecules according to the invention may also comprise modified fragments of antibodies, also called antibody variants, such as scFv, di-scFv or bi(s)-scFv, scFv-Fc, scFv-zipper, scFab, Fab_2, Fab_3, diabodies, single chain diabodies, tandem diabodies (Tandab's), tandem di-scFv, tandem tri-scFv, "multibodies" such as triabodies or tetrabodies, and single domain antibodies such as nanobodies or single variable domain antibodies comprising merely one variable domain, which might be VHH, VH or VL, that specifically bind an antigen or epitope independently of other V regions or domains.

[57] As used herein, the terms "single-chain Fv," "single-chain antibodies" or "scFv" refer to single polypeptide chain antibody fragments that comprise the variable regions from both the heavy and light chains, but lack the constant regions. Generally, a single-chain antibody further comprises a polypeptide linker between the VH and VL domains which enables it to form the desired structure which would allow for antigen binding. Single chain antibodies are discussed in detail by Pluckthun in The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994). Various methods of generating single chain antibodies are known, including those described in U.S. Pat. Nos. 4,694,778 and 5,260,203; International Patent Application Publication No. WO 88/01649; Bird (1988) Science 242:423-442; Huston *et al.* (1988) Proc. Natl. Acad. Sci. USA 85:5879-5883; Ward *et al.* (1989) Nature 334:54454; Skerra *et al.* (1988) Science 242:1038-1041. In specific embodiments, single-chain antibodies can also be bispecific, multispecific, human, and/or humanized and/or synthetic.

[58] Furthermore, the definition of the term "antigen-binding molecule" includes monovalent, bivalent and polyvalent / multivalent constructs and, thus, bispecific constructs, specifically binding to only two antigenic structure, as well as polyspecific / multispecific constructs, which specifically bind more than two antigenic structures, e.g. three, four or more, through distinct binding domains. Moreover, the definition of the term "antigen-binding molecule" includes molecules consisting of only one polypeptide chain as well as molecules consisting of more than one polypeptide chain, which chains can be either identical (homodimers, homotrimers or homo oligomers) or different (heterodimer, heterotrimer or heterooligomer). Examples for the above identified antibodies and variants or derivatives thereof are described *inter alia* in Harlow and Lane, Antibodies a laboratory manual, CSHL Press (1988) and Using Antibodies: a laboratory manual, CSHL Press (1999), Kontermann and Dübel, Antibody Engineering,

Springer, 2nd ed. 2010 and Little, Recombinant Antibodies for Immunotherapy, Cambridge University Press 2009.

[59] The term "polypeptide" as used herein describes a group of molecules, which usually consist of more than 30 amino acids. Polypeptides may further form multimers such as dimers, trimers and higher oligomers, i.e., consisting of more than one polypeptide molecule. Polypeptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding higher order structures of such multimers are, consequently, termed homo- or heterodimers, homo- or heterotrimers etc. An example for a heteromultimer is an antibody molecule, which, in its naturally occurring form, consists of two identical light polypeptide chains and two identical heavy polypeptide chains. The terms “peptide”, "polypeptide" and "protein" also refer to naturally modified peptides / polypeptides / proteins wherein the modification is effected e.g. by post-translational modifications like glycosylation, acetylation, phosphorylation and the like. A “peptide”, "polypeptide" or "protein" when referred to herein may also be chemically modified such as pegylated. Such modifications are well known in the art and described herein below.

[60] The term “bispecific” as used herein refers to an antigen-binding molecule which is “at least bispecific”, *i.e.*, it comprises at least a first binding domain and a second binding domain, wherein the first binding domain binds to one antigen or target (e.g. the target cell surface antigen), and the second binding domain binds to another antigen or target (e.g. CD3). Accordingly, antigen-binding molecules according to the invention comprise specificities for at least two different antigens or targets. For example, the first domain does preferably not bind to an extracellular epitope of CD3ε of one or more of the species as described herein. The term “target cell surface antigen” refers to an antigenic structure expressed by a cell and which is present at the cell surface such that it is accessible for an antigen-binding molecule as described herein. It may be a protein, preferably the extracellular portion of a protein, or a carbohydrate structure, preferably a carbohydrate structure of a protein, such as a glycoprotein. It is preferably a tumor antigen. The term “bispecific antigen-binding molecule” of the invention also encompasses multispecific antigen-binding molecules such as trispecific antigen-binding molecules, the latter ones including three binding domains, or constructs having more than three (e.g. four, five…) specificities.

[61] Given that the antigen-binding molecules according to the invention are (at least) bispecific, they do not occur naturally and they are markedly different from naturally occurring products. A “bispecific” antigen-binding molecule or immunoglobulin is hence an artificial hybrid antibody or immunoglobulin having at least two distinct binding sides with different specificities. Bispecific antigen-binding molecules can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, *e.g.*, Songsivilai & Lachmann, Clin. Exp. Immunol. 79:315-321 (1990).

[62] The at least two binding domains and the variable domains (VH / VL) of the antigen-binding molecule of the present invention may or may not comprise peptide linkers (spacer peptides). The term “peptide linker” comprises in accordance with the present invention an amino acid sequence by which the

amino acid sequences of one (variable and/or binding) domain and another (variable and/or binding) domain of the antigen-binding molecule of the invention are linked with each other. The peptide linkers can also be used to fuse the third domain to the other domains of the antigen-binding molecule of the invention. An essential technical feature of such peptide linker is that it does not comprise any polymerization activity. Among the suitable peptide linkers are those described in U.S. Patents 4,751,180 and 4,935,233 or WO 88/09344. The peptide linkers can also be used to attach other domains or modules or regions (such as half-life extending domains) to the antigen-binding molecule of the invention.

[63] The antigen-binding molecules of the present invention are preferably "*in vitro* generated antigen-binding molecules". This term refers to an antigen-binding molecule according to the above definition where all or part of the variable region (e.g., at least one CDR) is generated in a non-immune cell selection, *e.g.*, an *in vitro* phage display, protein chip or any other method in which candidate sequences can be tested for their ability to bind to an antigen. This term thus preferably excludes sequences generated solely by genomic rearrangement in an immune cell in an animal. A "recombinant antibody" is an antibody made through the use of recombinant DNA technology or genetic engineering.

[64] The term "monoclonal antibody" (mAb) or monoclonal antigen-binding molecule as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post-translation modifications (e.g., isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic side or determinant on the antigen, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (or epitopes). In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, hence uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method.

[65] For the preparation of monoclonal antibodies, any technique providing antibodies produced by continuous cell line cultures can be used. For example, monoclonal antibodies to be used may be made by the hybridoma method first described by Koehler *et al.*, Nature, 256: 495 (1975), or may be made by recombinant DNA methods (see, e.g., U.S. Patent No. 4,816,567). Examples for further techniques to produce human monoclonal antibodies include the trioma technique, the human B-cell hybridoma technique (Kozbor, Immunology Today 4 (1983), 72) and the EBV-hybridoma technique (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985), 77-96).

[66] Hybridomas can then be screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA) and surface plasmon resonance (BIACORE™) analysis, to identify one or more hybridomas that produce an antibody that specifically binds with a specified antigen. Any form of the

relevant antigen may be used as the immunogen, *e.g.*, recombinant antigen, naturally occurring forms, any variants or fragments thereof, as well as an antigenic peptide thereof. Surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to an epitope of a target cell surface antigen, (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmborg, J. Immunol. Methods 183 (1995), 7-13).

[67] Another exemplary method of making monoclonal antibodies includes screening protein expression libraries, e.g., phage display or ribosome display libraries. Phage display is described, for example, in Ladner *et al.*, U.S. Patent No. 5,223,409; Smith (1985) Science 228:1315-1317, Clackson *et al.*, Nature, 352: 624-628 (1991) and Marks *et al.*, J. Mol. Biol., 222: 581-597 (1991).

[68] In addition to the use of display libraries, the relevant antigen can be used to immunize a non-human animal, *e.g.*, a rodent (such as a mouse, hamster, rabbit or rat). In one embodiment, the non-human animal includes at least a part of a human immunoglobulin gene. For example, it is possible to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig (immunoglobulin) loci. Using the hybridoma technology, antigen-specific monoclonal antibodies derived from the genes with the desired specificity may be produced and selected. See, e.g., XENOMOUSE™, Green *et al.* (1994) Nature Genetics 7:13-21, US 2003-0070185, WO 96/34096, and WO 96/33735.

[69] A monoclonal antibody can also be obtained from a non-human animal, and then modified, *e.g.*, humanized, deimmunized, rendered chimeric etc., using recombinant DNA techniques known in the art. Examples of modified antigen-binding molecules include humanized variants of non-human antibodies, "affinity matured" antibodies (see, *e.g.* Hawkins et al. J. Mol. Biol. 254, 889-896 (1992) and Lowman *et al.*, Biochemistry 30, 10832- 10837 (1991)) and antibody mutants with altered effector function(s) (see, *e.g.*, US Patent 5,648,260, Kontermann and Dübel (2010), *loc. cit.* and Little (2009), *loc. cit.*).

[70] In immunology, affinity maturation is the process by which B cells produce antibodies with increased affinity for antigen during the course of an immune response. With repeated exposures to the same antigen, a host will produce antibodies of successively greater affinities. Like the natural prototype, the *in vitro* affinity maturation is based on the principles of mutation and selection. The *in vitro* affinity maturation has successfully been used to optimize antibodies, antigen-binding molecules, and antibody fragments. Random mutations inside the CDRs are introduced using radiation, chemical mutagens or error-prone PCR. In addition, the genetic diversity can be increased by chain shuffling. Two or three rounds of mutation and selection using display methods like phage display usually results in antibody fragments with affinities in the low nanomolar range.

[71] A preferred type of an amino acid substitutional variation of the antigen-binding molecules involves substituting one or more hypervariable region residues of a parent antibody (e. g. a humanized or human antibody). Generally, the resulting variant(s) selected for further development will have improved

biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sides (e. g. 6-7 sides) are mutated to generate all possible amino acid substitutions at each side. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e. g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sides for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the binding domain and, *e.g.*, human target cell surface antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[72] The monoclonal antibodies and antigen-binding molecules of the present invention specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is/are identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U.S. Patent No. 4,816,567; Morrison et al., Proc. Natl. Acad. Sci. USA, 81: 6851-6855 (1984)). Chimeric antibodies of interest herein include "primitized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey, Ape etc.) and human constant region sequences. A variety of approaches for making chimeric antibodies have been described. See *e.g.*, Morrison *et al.*, Proc. Natl. Acad. Sci U.S.A. 81:6851 , 1985; Takeda *et al.*, Nature 314:452, 1985, Cabilly *et al.*, U.S. Patent No. 4,816,567; Boss *et al.*, U.S. Patent No. 4,816,397; Tanaguchi *et al.*, EP 0171496; EP 0173494; and GB 2177096.

[73] An antibody, antigen-binding molecule, antibody fragment or antibody variant may also be modified by specific deletion of human T cell epitopes (a method called "deimmunization") by the methods disclosed for example in WO 98/52976 or WO 00/34317. Briefly, the heavy and light chain variable domains of an antibody can be analyzed for peptides that bind to MHC class II; these peptides represent potential T cell epitopes (as defined in WO 98/52976 and WO 00/34317). For detection of potential T cell epitopes, a computer modeling approach termed "peptide threading" can be applied, and in addition a database of human MHC class Il binding peptides can be searched for motifs present in the VH and VL sequences, as described in WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class Il DR allotypes, and thus constitute potential T cell epitopes. Potential T cell epitopes detected

can be eliminated by substituting small numbers of amino acid residues in the variable domains, or preferably, by single amino acid substitutions. Typically, conservative substitutions are made. Often, but not exclusively, an amino acid common to a position in human germline antibody sequences may be used. Human germline sequences are disclosed *e.g.* in Tomlinson, *et al.* (1992) J. MoI. Biol. 227:776-798; Cook, G.P. *et al.* (1995) Immunol. Today Vol. 16 (5): 237-242; and Tomlinson et al. (1995) EMBO J. 14: 14:4628-4638. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, LA. *et al.* MRC Centre for Protein Engineering, Cambridge, UK). These sequences can be used as a source of human sequence, *e.g.*, for framework regions and CDRs. Consensus human framework regions can also be used, for example as described in US Patent No. 6,300,064.

[74] "Humanized" antibodies, antigen-binding molecules, variants or fragments thereof (such as Fv, Fab, Fab', F(ab')2 or other antigen-binding subsequences of antibodies) are antibodies or immunoglobulins of mostly human sequences, which contain (a) minimal sequence(s) derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (also CDR) of the recipient are replaced by residues from a hypervariable region of a non-human (*e.g.*, rodent) species (donor antibody) such as mouse, rat, hamster or rabbit having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, "humanized antibodies" as used herein may also comprise residues which are found neither in the recipient antibody nor the donor antibody. These modifications are made to further refine and optimize antibody performance. The humanized antibody may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, Nature, 321: 522-525 (1986); Reichmann *et al.*, Nature, 332: 323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2: 593-596 (1992).

[75] Humanized antibodies or fragments thereof can be generated by replacing sequences of the Fv variable domain that are not directly involved in antigen binding with equivalent sequences from human Fv variable domains. Exemplary methods for generating humanized antibodies or fragments thereof are provided by Morrison (1985) Science 229:1202-1207; by Oi *et al.* (1986) BioTechniques 4:214; and by US 5,585,089; US 5,693,761; US 5,693,762; US 5,859,205; and US 6,407,213. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable domains from at least one of a heavy or light chain. Such nucleic acids may be obtained from a hybridoma producing an antibody against a predetermined target, as described above, as well as from other sources. The recombinant DNA encoding the humanized antibody molecule can then be cloned into an appropriate expression vector.

[76] Humanized antibodies may also be produced using transgenic animals such as mice that express human heavy and light chain genes, but are incapable of expressing the endogenous mouse

immunoglobulin heavy and light chain genes. Winter describes an exemplary CDR grafting method that may be used to prepare the humanized antibodies described herein (U.S. Patent No. 5,225,539). All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR, or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

[77] A humanized antibody can be optimized by the introduction of conservative substitutions, consensus sequence substitutions, germline substitutions and/or back mutations. Such altered immunoglobulin molecules can be made by any of several techniques known in the art, (*e.g.*, Teng *et al.*, Proc. Natl. Acad. Sci. U.S.A., 80: 7308-7312, 1983; Kozbor *et al.*, Immunology Today, 4: 7279, 1983; Olsson *et al.*, Meth. Enzymol., 92: 3-16, 1982, and EP 239 400).

[78] The term "human antibody", "human antigen-binding molecule" and "human binding domain" includes antibodies, antigen-binding molecules and binding domains having antibody regions such as variable and constant regions or domains which correspond substantially to human germline immunoglobulin sequences known in the art, including, for example, those described by Kabat *et al.* (1991) (*loc. cit.*). The human antibodies, antigen-binding molecules or binding domains of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (*e.g.*, mutations introduced by random or side-specific mutagenesis *in vitro* or by somatic mutation *in vivo*), for example in the CDRs, and in particular, in CDR3. The human antibodies, antigen-binding molecules or binding domains can have at least one, two, three, four, five, or more positions replaced with an amino acid residue that is not encoded by the human germline immunoglobulin sequence. The definition of human antibodies, antigen-binding molecules and binding domains as used herein, however, also contemplates "fully human antibodies", which include only non-artificially and/or genetically altered human sequences of antibodies as those can be derived by using technologies or systems such as the Xenomouse.Preferably, a "fully human antibody" does not include amino acid residues not encoded by human germline immunoglobulin sequences

[79] In some embodiments, the antigen-binding molecules of the invention are "isolated" or "substantially pure" antigen-binding molecules. "Isolated" or "substantially pure", when used to describe the antigen-binding molecules disclosed herein, means an antigen-binding molecule that has been identified, separated and/or recovered from a component of its production environment. Preferably, the antigen-binding molecule is free or substantially free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. The antigen-binding molecules may e.g constitute at least about 5%, or at least about 50% by weight of the total protein in a given sample. It is understood that the isolated protein may constitute from 5% to 99.9% by weight of the total protein content, depending on the circumstances. The

polypeptide may be made at a significantly higher concentration through the use of an inducible promoter or high expression promoter, such that it is made at increased concentration levels. The definition includes the production of an antigen-binding molecule in a wide variety of organisms and/or host cells that are known in the art. In preferred embodiments, the antigen-binding molecule will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Ordinarily, however, an isolated antigen-binding molecule will be prepared by at least one purification step.

[80] The term "binding domain" characterizes in connection with the present invention a domain which (specifically) binds to / interacts with / recognizes a given target epitope or a given target side on the target molecules (antigens), e.g. CD33 and CD3, respectively. The structure and function of the first binding domain (recognizing e.g. CD33), and preferably also the structure and/or function of the second binding domain (recognizing e.g. CD3), is/are based on the structure and/or function of an antibody, e.g. of a full-length or whole immunoglobulin molecule and/or is/are drawn from the variable heavy chain (VH) and/or variable light chain (VL) domains of an antibody or fragment thereof. Preferably the first binding domain is characterized by the presence of three light chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VL region) and/or three heavy chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VH region). The second binding domain preferably also comprises the minimum structural requirements of an antibody which allow for the target binding. More preferably, the second binding domain comprises at least three light chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VL region) and/or three heavy chain CDRs (i.e. CDR1, CDR2 and CDR3 of the VH region). It is envisaged that the first and/or second binding domain is produced by or obtainable by phage-display or library screening methods rather than by grafting CDR sequences from a pre-existing (monoclonal) antibody into a scaffold.

[81] According to the present invention, binding domains are in the form of one or more polypeptides. Such polypeptides may include proteinaceous parts and non-proteinaceous parts (e.g. chemical linkers or chemical cross-linking agents such as glutaraldehyde). Proteins (including fragments thereof, preferably biologically active fragments, and peptides, usually having less than 30 amino acids) comprise two or more amino acids coupled to each other via a covalent peptide bond (resulting in a chain of amino acids).

[82] The term "polypeptide" as used herein describes a group of molecules, which usually consist of more than 30 amino acids. Polypeptides may further form multimers such as dimers, trimers and higher oligomers, *i.e.*, consisting of more than one polypeptide molecule. Polypeptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding higher order structures of such multimers are, consequently, termed homo- or heterodimers, homo- or heterotrimers etc. An example for a heteromultimer is an antibody molecule, which, in its naturally occurring form, consists of two identical light polypeptide chains and two identical heavy polypeptide chains. The terms “peptide”, "polypeptide" and "protein" also refer to naturally modified peptides / polypeptides / proteins wherein the modification

is effected *e.g.* by post-translational modifications like glycosylation, acetylation, phosphorylation and the like. A “peptide”, "polypeptide" or "protein" when referred to herein may also be chemically modified such as pegylated. Such modifications are well known in the art and described herein below.

[83] Preferably the binding domain which binds to the target cell surface antigen and/or the binding domain which binds to CD3ε is/are human binding domains. Antibodies and antigen-binding molecules comprising at least one human binding domain avoid some of the problems associated with antibodies or antigen-binding molecules that possess non-human such as rodent (*e.g.* murine, rat, hamster or rabbit) variable and/or constant regions. The presence of such rodent derived proteins can lead to the rapid clearance of the antibodies or antigen-binding molecules or can lead to the generation of an immune response against the antibody or antigen-binding molecule by a patient. In order to avoid the use of rodent derived antibodies or antigen-binding molecules, human or fully human antibodies / antigen-binding molecules can be generated through the introduction of human antibody function into a rodent so that the rodent produces fully human antibodies.

[84] The ability to clone and reconstruct megabase-sized human loci in YACs and to introduce them into the mouse germline provides a powerful approach to elucidating the functional components of very large or crudely mapped loci as well as generating useful models of human disease. Furthermore, the use of such technology for substitution of mouse loci with their human equivalents could provide unique insights into the expression and regulation of human gene products during development, their communication with other systems, and their involvement in disease induction and progression.

[85] An important practical application of such a strategy is the “humanization” of the mouse humoral immune system. Introduction of human immunoglobulin (Ig) loci into mice in which the endogenous Ig genes have been inactivated offers the opportunity to study the mechanisms underlying programmed expression and assembly of antibodies as well as their role in B-cell development. Furthermore, such a strategy could provide an ideal source for production of fully human monoclonal antibodies (mAbs) – an important milestone towards fulfilling the promise of antibody therapy in human disease. Fully human antibodies or antigen-binding molecules are expected to minimize the immunogenic and allergic responses intrinsic to mouse or mouse-derivatized mAbs and thus to increase the efficacy and safety of the administered antibodies / antigen-binding molecules. The use of fully human antibodies or antigen-binding molecules can be expected to provide a substantial advantage in the treatment of chronic and recurring human diseases, such as inflammation, autoimmunity, and cancer, which require repeated compound administrations.

[86] One approach towards this goal was to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci in anticipation that such mice would produce a large repertoire of human antibodies in the absence of mouse antibodies. Large human Ig fragments would preserve the large variable gene diversity as well as the proper regulation of antibody production and

expression. By exploiting the mouse machinery for antibody diversification and selection and the lack of immunological tolerance to human proteins, the reproduced human antibody repertoire in these mouse strains should yield high affinity antibodies against any antigen of interest, including human antigens. Using the hybridoma technology, antigen-specific human mAbs with the desired specificity could be readily produced and selected. This general strategy was demonstrated in connection with the generation of the first XenoMouse mouse strains (see Green et al. Nature Genetics 7:13-21 (1994)). The XenoMouse strains were engineered with yeast artificial chromosomes (YACs) containing 245 kb and 190 kb-sized germline configuration fragments of the human heavy chain locus and kappa light chain locus, respectively, which contained core variable and constant region sequences. The human Ig containing YACs proved to be compatible with the mouse system for both rearrangement and expression of antibodies and were capable of substituting for the inactivated mouse Ig genes. This was demonstrated by their ability to induce B cell development, to produce an adult-like human repertoire of fully human antibodies, and to generate antigen-specific human mAbs. These results also suggested that introduction of larger portions of the human Ig loci containing greater numbers of V genes, additional regulatory elements, and human Ig constant regions might recapitulate substantially the full repertoire that is characteristic of the human humoral response to infection and immunization. The work of Green et al. was recently extended to the introduction of greater than approximately 80% of the human antibody repertoire through introduction of megabase sized, germline configuration YAC fragments of the human heavy chain loci and kappa light chain loci, respectively. See Mendez *et al.* Nature Genetics 15:146-156 (1997) and U.S. patent application Ser. No. 08/759,620.

[87] The production of the XenoMouse mice is further discussed and delineated in U.S. patent applications Ser. No. 07/466,008, Ser. No. 07/610,515, Ser. No. 07/919,297, Ser. No. 07/922,649, Ser. No. 08/031,801, Ser. No. 08/112,848, Ser. No. 08/234,145, Ser. No. 08/376,279, Ser. No. 08/430,938, Ser. No. 08/464,584, Ser. No. 08/464,582, Ser. No. 08/463,191, Ser. No. 08/462,837, Ser. No. 08/486,853, Ser. No. 08/486,857, Ser. No. 08/486,859, Ser. No. 08/462,513, Ser. No. 08/724,752, and Ser. No. 08/759,620; and U.S. Pat. Nos. 6,162,963; 6,150,584; 6,114,598; 6,075,181, and 5,939,598 and Japanese Patent Nos. 3 068 180 B2, 3 068 506 B2, and 3 068 507 B2. See also Mendez *et al.* Nature Genetics 15:146-156 (1997) and Green and Jakobovits J. Exp. Med. 188:483-495 (1998), EP 0 463 151 B1, WO 94/02602, WO 96/34096, WO 98/24893, WO 00/76310, and WO 03/47336.

[88] In an alternative approach, others, including GenPharm International, Inc., have utilized a "minilocus" approach. In the minilocus approach, an exogenous Ig locus is mimicked through the inclusion of pieces (individual genes) from the Ig locus. Thus, one or more VH genes, one or more DH genes, one or more JH genes, a mu constant region, and a second constant region (preferably a gamma constant region) are formed into a construct for insertion into an animal. This approach is described in U.S. Pat. No. 5,545,807 to Surani *et al.* and U.S. Pat. Nos. 5,545,806; 5,625,825; 5,625,126; 5,633,425;

5,661,016; 5,770,429; 5,789,650; 5,814,318; 5,877,397; 5,874,299; and 6,255,458 each to Lonberg and Kay, U.S. Pat. Nos. 5,591,669 and 6,023.010 to Krimpenfort and Berns, U.S. Pat. Nos. 5,612,205; 5,721,367; and 5,789,215 to Berns *et al.*, and U.S. Pat. No. 5,643,763 to Choi and Dunn, and GenPharm International U.S. patent application Ser. No. 07/574,748, Ser. No. 07/575,962, Ser. No. 07/810,279, Ser. No. 07/853,408, Ser. No. 07/904,068, Ser. No. 07/990,860, Ser. No. 08/053,131, Ser. No. 08/096,762, Ser. No. 08/155,301, Ser. No. 08/161,739, Ser. No. 08/165,699, Ser. No. 08/209,741. See also EP 0 546 073 B1, WO 92/03918, WO 92/22645, WO 92/22647, WO 92/22670, WO 93/12227, WO 94/00569, WO 94/25585, WO 96/14436, WO 97/13852, and WO 98/24884 and U.S. Pat. No. 5,981,175. See further Taylor *et al.* (1992), Chen *et al.* (1993), Tuaillon *et al.* (1993), Choi *et al.* (1993), Lonberg *et al.* (1994), Taylor *et al.* (1994), and Tuaillon *et al.* (1995), Fishwild *et al.* (1996).

[89] Kirin has also demonstrated the generation of human antibodies from mice in which, through microcell fusion, large pieces of chromosomes, or entire chromosomes, have been introduced. See European Patent Application Nos. 773 288 and 843 961. Xenerex Biosciences is developing a technology for the potential generation of human antibodies. In this technology, SCID mice are reconstituted with human lymphatic cells, e.g., B and/or T cells. Mice are then immunized with an antigen and can generate an immune response against the antigen. See U.S. Pat. Nos. 5,476,996; 5,698,767; and 5,958,765.

[90] Human anti-mouse antibody (HAMA) responses have led the industry to prepare chimeric or otherwise humanized antibodies. It is however expected that certain human anti-chimeric antibody (HACA) responses will be observed, particularly in chronic or multi-dose utilizations of the antibody. Thus, it would be desirable to provide antigen-binding molecules comprising a human binding domain against the target cell surface antigen and a human binding domain against CD3ε in order to vitiate concerns and/or effects of HAMA or HACA response.

[91] The terms "(specifically) binds to", (specifically) recognizes", "is (specifically) directed to", and "(specifically) reacts with" mean in accordance with this invention that a binding domain interacts or specifically interacts with a given epitope or a given target side on the target molecules (antigens), here: target cell surface antigen and CD3ε, respectively.

[92] The term "epitope" refers to a side on an antigen to which a binding domain, such as an antibody or immunoglobulin, or a derivative, fragment or variant of an antibody or an immunoglobulin, specifically binds. An "epitope" is antigenic and thus the term epitope is sometimes also referred to herein as "antigenic structure" or "antigenic determinant". Thus, the binding domain is an "antigen interaction side". Said binding/interaction is also understood to define a "specific recognition".

[93] "Epitopes" can be formed both by contiguous amino acids or non-contiguous amino acids juxtaposed by tertiary folding of a protein. A "linear epitope" is an epitope where an amino acid primary

sequence comprises the recognized epitope. A linear epitope typically includes at least 3 or at least 4, and more usually, at least 5 or at least 6 or at least 7, for example, about 8 to about 10 amino acids in a unique sequence.

[94] A "conformational epitope", in contrast to a linear epitope, is an epitope wherein the primary sequence of the amino acids comprising the epitope is not the sole defining component of the epitope recognized (*e.g.*, an epitope wherein the primary sequence of amino acids is not necessarily recognized by the binding domain). Typically a conformational epitope comprises an increased number of amino acids relative to a linear epitope. With regard to recognition of conformational epitopes, the binding domain recognizes a three-dimensional structure of the antigen, preferably a peptide or protein or fragment thereof (in the context of the present invention, the antigenic structure for one of the binding domains is comprised within the target cell surface antigen protein). For example, when a protein molecule folds to form a three-dimensional structure, certain amino acids and/or the polypeptide backbone forming the conformational epitope become juxtaposed enabling the antibody to recognize the epitope. Methods of determining the conformation of epitopes include, but are not limited to, x-ray crystallography, two-dimensional nuclear magnetic resonance (2D-NMR) spectroscopy and site-directed spin labelling and electron paramagnetic resonance (EPR) spectroscopy.

[95] A method for epitope mapping is described in the following: When a region (a contiguous amino acid stretch) in the human target cell surface antigen protein is exchanged / replaced with its corresponding region of a non-human and non-primate target cell surface antigen (*e.g.*, mouse target cell surface antigen, but others like chicken, rat, hamster, rabbit etc. might also be conceivable), a decrease in the binding of the binding domain is expected to occur, unless the binding domain is cross-reactive for the non-human, non-primate target cell surface antigen used. Said decrease is preferably at least 10%, 20%, 30%, 40%, or 50%; more preferably at least 60%, 70%, or 80%, and most preferably 90%, 95% or even 100% in comparison to the binding to the respective region in the human target cell surface antigen protein, whereby binding to the respective region in the human target cell surface antigen protein is set to be 100%. It is envisaged that the aforementioned human target cell surface antigen / non-human target cell surface antigen chimeras are expressed in CHO cells. It is also envisaged that the human target cell surface antigen / non-human target cell surface antigen chimeras are fused with a transmembrane domain and/or cytoplasmic domain of a different membrane-bound protein such as EpCAM.

[96] In an alternative or additional method for epitope mapping, several truncated versions of the human target cell surface antigen extracellular domain can be generated in order to determine a specific region that is recognized by a binding domain. In these truncated versions, the different extracellular target cell surface antigen domains / sub-domains or regions are stepwise deleted, starting from the N-terminus. It is envisaged that the truncated target cell surface antigen versions may be expressed in CHO cells. It is also envisaged that the truncated target cell surface antigen versions may be fused with a transmembrane domain and/or cytoplasmic domain of a different membrane-bound protein such as EpCAM. It is also

envisaged that the truncated target cell surface antigen versions may encompass a signal peptide domain at their N-terminus, for example a signal peptide derived from mouse IgG heavy chain signal peptide. It is furthermore envisaged that the truncated target cell surface antigen versions may encompass a v5 domain at their N-terminus (following the signal peptide) which allows verifying their correct expression on the cell surface. A decrease or a loss of binding is expected to occur with those truncated target cell surface antigen versions which do not encompass any more the target cell surface antigen region that is recognized by the binding domain. The decrease of binding is preferably at least 10%, 20%, 30%, 40%, 50%; more preferably at least 60%, 70%, 80%, and most preferably 90%, 95% or even 100%, whereby binding to the entire human target cell surface antigen protein (or its extracellular region or domain) is set to be 100.

[97] A further method to determine the contribution of a specific residue of a target cell surface antigen to the recognition by an antigen-binding molecule or binding domain is alanine scanning (see e.g. Morrison KL & Weiss GA. Cur Opin Chem Biol. 2001 Jun;5(3):302-7), where each residue to be analyzed is replaced by alanine, e.g. via site-directed mutagenesis. Alanine is used because of its non-bulky, chemically inert, methyl functional group that nevertheless mimics the secondary structure references that many of the other amino acids possess. Sometimes bulky amino acids such as valine or leucine can be used in cases where conservation of the size of mutated residues is desired. Alanine scanning is a mature technology which has been used for a long period of time.

[98] The interaction between the binding domain and the epitope or the region comprising the epitope implies that a binding domain exhibits appreciable affinity for the epitope / the region comprising the epitope on a particular protein or antigen (here: target cell surface antigen and CD3, respectively) and, generally, does not exhibit significant reactivity with proteins or antigens other than the target cell surface antigen or CD3. "Appreciable affinity" includes binding with an affinity of about 10^{-6} M (KD) or stronger. Preferably, binding is considered specific when the binding affinity is about 10^{-12} to 10^{-8} M, 10^{-12} to 10^{-9} M, 10^{-12} to 10^{-10} M, 10^{-11} to 10^{-8} M, preferably of about 10^{-11} to 10^{-9} M. Whether a binding domain specifically reacts with or binds to a target can be tested readily by, *inter alia*, comparing the reaction of said binding domain with a target protein or antigen with the reaction of said binding domain with proteins or antigens other than the target cell surface antigen or CD3. Preferably, a binding domain of the invention does not essentially or substantially bind to proteins or antigens other than the target cell surface antigen or CD3 (*i.e.*, the first binding domain is preferably not capable of binding to proteins other than the target cell surface antigen and the second binding domain is not capable of binding to proteins other than CD3). It is an envisaged characteristic of the antigen-binding molecules according to the present invention to have superior affinity characteristics in comparison to other HLE formats. Such a superior affinity, in consequence, suggests a prolonged half-life in vivo. The longer half-life of the antigen-binding molecules according to the present invention may reduce the duration and frequency of administration which typically contributes to improved patient compliance. This is of particular importance as the antigen-binding

molecules of the present invention are particularly beneficial for highly weakened or even multimorbide cancer patients.

[99] The term "does not essentially / substantially bind" or "is not capable of binding" means that a binding domain of the present invention does not bind a protein or antigen other than the target cell surface antigen or CD3, *i.e.*, does not show reactivity of more than 30%, preferably not more than 20%, more preferably not more than 10%, particularly preferably not more than 9%, 8%, 7%, 6% or 5% with proteins or antigens other than the target cell surface antigen or CD3, whereby binding to the target cell surface antigen or CD3, respectively, is set to be 100%.

[100] Specific binding is believed to be effected by specific motifs in the amino acid sequence of the binding domain and the antigen. Thus, binding is achieved as a result of their primary, secondary and/or tertiary structure as well as the result of secondary modifications of said structures. The specific interaction of the antigen-interaction-side with its specific antigen may result in a simple binding of said side to the antigen. Moreover, the specific interaction of the antigen-interaction-side with its specific antigen may alternatively or additionally result in the initiation of a signal, *e.g.* due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc.

[101] The term "variable" refers to the portions of the antibody or immunoglobulin domains that exhibit variability in their sequence and that are involved in determining the specificity and binding affinity of a particular antibody (i.e., the "variable domain(s)"). The pairing of a variable heavy chain (VH) and a variable light chain (VL) together forms a single antigen-binding side.

[102] Variability is not evenly distributed throughout the variable domains of antibodies; it is concentrated in sub-domains of each of the heavy and light chain variable regions. These sub-domains are called "hypervariable regions" or "complementarity determining regions" (CDRs). The more conserved (i.e., non-hypervariable) portions of the variable domains are called the "framework" regions (FRM or FR) and provide a scaffold for the six CDRs in three dimensional space to form an antigen-binding surface. The variable domains of naturally occurring heavy and light chains each comprise four FRM regions (FR1, FR2, FR3, and FR4), largely adopting a β-sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part of, the β-sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRM and, with the hypervariable regions from the other chain, contribute to the formation of the antigen-binding side (see Kabat *et al.*, *loc. cit.*).

[103] The terms "CDR", and its plural "CDRs", refer to the complementarity determining region of which three make up the binding character of a light chain variable region (CDR-L1, CDR-L2 and CDR-L3) and three make up the binding character of a heavy chain variable region (CDR-H1, CDR-H2 and CDR-H3). CDRs contain most of the residues responsible for specific interactions of the antibody with the

antigen and hence contribute to the functional activity of an antibody molecule: they are the main determinants of antigen specificity.

[104] The exact definitional CDR boundaries and lengths are subject to different classification and numbering systems. CDRs may therefore be referred to by Kabat, Chothia, contact or any other boundary definitions, including the numbering system described herein. Despite differing boundaries, each of these systems has some degree of overlap in what constitutes the so called "hypervariable regions" within the variable sequences. CDR definitions according to these systems may therefore differ in length and boundary areas with respect to the adjacent framework region. See for example Kabat (an approach based on cross-species sequence variability), Chothia (an approach based on crystallographic studies of antigen-antibody complexes), and/or MacCallum (Kabat *et al.*, *loc. cit.*; Chothia *et al.*, J. MoI. Biol, 1987, 196: 901-917; and MacCallum *et al.*, J. MoI. Biol, 1996, 262: 732). Still another standard for characterizing the antigen binding side is the AbM definition used by Oxford Molecular's AbM antibody modeling software. See, *e.g.*, Protein Sequence and Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg). To the extent that two residue identification techniques define regions of overlapping, but not identical regions, they can be combined to define a hybrid CDR. However, the numbering in accordance with the so-called Kabat system is preferred.

[105] Typically, CDRs form a loop structure that can be classified as a canonical structure. The term "canonical structure" refers to the main chain conformation that is adopted by the antigen binding (CDR) loops. From comparative structural studies, it has been found that five of the six antigen binding loops have only a limited repertoire of available conformations. Each canonical structure can be characterized by the torsion angles of the polypeptide backbone. Correspondent loops between antibodies may, therefore, have very similar three dimensional structures, despite high amino acid sequence variability in most parts of the loops (Chothia and Lesk, J. MoI. Biol., 1987, 196: 901; Chothia *et al.*, Nature, 1989, 342: 877; Martin and Thornton, J. MoI. Biol, 1996, 263: 800). Furthermore, there is a relationship between the adopted loop structure and the amino acid sequences surrounding it. The conformation of a particular canonical class is determined by the length of the loop and the amino acid residues residing at key positions within the loop, as well as within the conserved framework (*i.e.*, outside of the loop). Assignment to a particular canonical class can therefore be made based on the presence of these key amino acid residues.

[106] The term "canonical structure" may also include considerations as to the linear sequence of the antibody, for example, as catalogued by Kabat (Kabat *et al.*, loc. cit.). The Kabat numbering scheme (system) is a widely adopted standard for numbering the amino acid residues of an antibody variable domain in a consistent manner and is the preferred scheme applied in the present invention as also mentioned elsewhere herein. Additional structural considerations can also be used to determine the canonical structure of an antibody. For example, those differences not fully reflected by Kabat numbering can be described by the numbering system of Chothia *et al.* and/or revealed by other techniques, for

example, crystallography and two- or three-dimensional computational modeling. Accordingly, a given antibody sequence may be placed into a canonical class which allows for, among other things, identifying appropriate chassis sequences (*e.g.*, based on a desire to include a variety of canonical structures in a library). Kabat numbering of antibody amino acid sequences and structural considerations as described by Chothia *et al.*, *loc. cit.* and their implications for construing canonical aspects of antibody structure, are described in the literature. The subunit structures and three-dimensional configurations of different classes of immunoglobulins are well known in the art. For a review of the antibody structure, see Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, eds. Harlow *et al.*, 1988.

[107] The CDR3 of the light chain and, particularly, the CDR3 of the heavy chain may constitute the most important determinants in antigen binding within the light and heavy chain variable regions. In some antigen-binding molecules, the heavy chain CDR3 appears to constitute the major area of contact between the antigen and the antibody. *In vitro* selection schemes in which CDR3 alone is varied can be used to vary the binding properties of an antibody or determine which residues contribute to the binding of an antigen. Hence, CDR3 is typically the greatest source of molecular diversity within the antibody-binding side. H3, for example, can be as short as two amino acid residues or greater than 26 amino acids.

[108] In a classical full-length antibody or immunoglobulin, each light (L) chain is linked to a heavy (H) chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. The CH domain most proximal to VH is usually designated as CH1. The constant ("C") domains are not directly involved in antigen binding, but exhibit various effector functions, such as antibody-dependent, cell-mediated cytotoxicity and complement activation. The Fc region of an antibody is comprised within the heavy chain constant domains and is for example able to interact with cell surface located Fc receptors.

[109] The sequence of antibody genes after assembly and somatic mutation is highly varied, and these varied genes are estimated to encode 10^{10} different antibody molecules (Immunoglobulin Genes, 2nd ed., eds. Jonio et al., Academic Press, San Diego, CA, 1995). Accordingly, the immune system provides a repertoire of immunoglobulins. The term "repertoire" refers to at least one nucleotide sequence derived wholly or partially from at least one sequence encoding at least one immunoglobulin. The sequence(s) may be generated by rearrangement *in vivo* of the V, D, and J segments of heavy chains, and the V and J segments of light chains. Alternatively, the sequence(s) can be generated from a cell in response to which rearrangement occurs, e.g., *in vitro* stimulation. Alternatively, part or all of the sequence(s) may be obtained by DNA splicing, nucleotide synthesis, mutagenesis, and other methods, see, e.g., U.S. Patent 5,565,332. A repertoire may include only one sequence or may include a plurality of sequences, including ones in a genetically diverse collection.

[110] The term "Fc portion" or "Fc monomer" means in connection with this invention a polypeptide comprising at least one domain having the function of a CH2 domain and at least one domain having the

function of a CH3 domain of an immunoglobulin molecule. As apparent from the term "Fc monomer", the polypeptide comprising those CH domains is a "polypeptide monomer". An Fc monomer can be a polypeptide comprising at least a fragment of the constant region of an immunoglobulin excluding the first constant region immunoglobulin domain of the heavy chain (CH1), but maintaining at least a functional part of one CH2 domain and a functional part of one CH3 domain, wherein the CH2 domain is amino terminal to the CH3 domain. In a preferred aspect of this definition, an Fc monomer can be a polypeptide constant region comprising a portion of the Ig-Fc hinge region, a CH2 region and a CH3 region, wherein the hinge region is amino terminal to the CH2 domain. It is envisaged that the hinge region of the present invention promotes dimerization. Such Fc polypeptide molecules can be obtained by papain digestion of an immunoglobulin region (of course resulting in a dimer of two Fc polypeptide), for example and not limitation. In another aspect of this definition, an Fc monomer can be a polypeptide region comprising a portion of a CH2 region and a CH3 region. Such Fc polypeptide molecules can be obtained by pepsin digestion of an immunoglobulin molecule, for example and not limitation. In one embodiment, the polypeptide sequence of an Fc monomer is substantially similar to an Fc polypeptide sequence of: an IgG_1 Fc region, an IgG_2 Fc region, an IgG_3 Fc region, an IgG_4 Fc region, an IgM Fc region, an IgA Fc region, an IgD Fc region and an IgE Fc region. *(See, e.g.,* Padlan, Molecular Immunology, 31(3), 169-217 (1993)). Because there is some variation between immunoglobulins, and solely for clarity, Fc monomer refers to the last two heavy chain constant region immunoglobulin domains of IgA, IgD, and IgG, and the last three heavy chain constant region immunoglobulin domains of IgE and IgM. As mentioned, the Fc monomer can also include the flexible hinge N-terminal to these domains. For IgA and IgM, the Fc monomer may include the J chain. For IgG, the Fc portion comprises immunoglobulin domains CH2 and CH3 and the hinge between the first two domains and CH2. Although the boundaries of the Fc portion may vary an example for a human IgG heavy chain Fc portion comprising a functional hinge, CH2 and CH3 domain can be defined e.g. to comprise residues D231 (of the hinge domain – corresponding to D234 in Table 1 below)) to P476, respectively L476 (for IgG_4) of the carboxyl-terminus of the CH3 domain, wherein the numbering is according to Kabat. The two Fc portions or Fc monomers, which are fused to each other via a peptide linker define the third domain of the antigen-binding molecule of the invention, which may also be defined as scFc domain.

[111] In one embodiment of the invention it is envisaged that a scFc domain as disclosed herein, respectively the Fc monomers fused to each other are comprised only in the third domain of the antigen-binding molecule.

In line with the present invention an IgG hinge region can be identified by analogy using the Kabat numbering as set forth in Table 1. In line with the above, it is envisaged that a hinge domain/region of the present invention comprises the amino acid residues corresponding to the IgG_1 sequence stretch of D234 to P243 according to the Kabat numbering. It is likewise envisaged that a hinge domain/region of the present invention comprises or consists of the IgG1 hinge sequence DKTHTCPPCP (SEQ ID NO: 182)

(corresponding to the stretch D234 to P243 as shown in Table 1 below – variations of said sequence are also envisaged provided that the hinge region still promotes dimerization). In a preferred embodiment of the invention the glycosylation site at Kabat position 314 of the CH2 domains in the third domain of the antigen-binding molecule is removed by a N314X substitution, wherein X is any amino acid excluding Q. Said substitution is preferably a N314G substitution. In a more preferred embodiment, said CH2 domain additionally comprises the following substitutions (position according to Kabat) V321C and R309C (these substitutions introduce the intra domain cysteine disulfide bridge at Kabat positions 309 and 321).

It is also envisaged that the third domain of the antigen-binding molecule of the invention comprises or consists in an amino to carboxyl order: DKTHTCPPCP (SEQ ID NO: 182) (i.e. hinge) –CH2-CH3-linker-DKTHTCPPCP (SEQ ID NO: 182) (i.e. hinge) –CH2-CH3. The peptide linker of the aforementioned antigen-binding molecule is in a preferred embodiment characterized by the amino acid sequence Gly-Gly-Gly-Gly-Ser, i.e. Gly_4Ser (SEQ ID NO: 187), or polymers thereof, i.e. $(Gly_4Ser)x$, where x is an integer of 5 or greater (e.g. 5, 6, 7, 8 etc. or greater), 6 being preferred ((Gly4Ser)6). Said construct may further comprise the aforementioned substitutions N314X, preferably N314G, and/or the further substitutions V321C and R309C. In a preferred embodiment of the antigen-binding molecules of the invention as defined herein before, it is envisaged that the second domain binds to an extracellular epitope of the human and/or the *Macaca* CD3ε chain.

Table 2: Kabat numbering of the amino acid residues of the hinge region

IMGT numbering for the hinge	IgG_1 amino acid translation	Kabat numbering
1	I	226
2	P	227
3	K	228
4	S	232
5	C	233
6	D	234
7	K	235
8	T	236
9	H	237
10	T	238
11	C	239
12	P	240
13	P	241
14	C	242
15	P	243

In further embodiments of the present invention, the hinge domain/region comprises or consists of the IgG2 subtype hinge sequence ERKCCVECPPCP (SEQ ID NO: 183), the IgG3 subtype hinge sequence

ELKTPLDTTHTCPRCP (SEQ ID NO: 184) or ELKTPLGDTTHTCPRCP (SEQ ID NO: 185), and/or the IgG4 subtype hinge sequence ESKYGPPCPSCP (SEQ ID NO: 186). The IgG1 subtype hinge sequence may be the following one EPKSCDKTHTCPPCP (as shown in Table 1 and SEQ ID NO: 183). These core hinge regions are thus also envisaged in the context of the present invention.

[112] The location and sequence of the IgG CH2 and IgG CD3 domain can be identified by analogy using the Kabat numbering as set forth in Table 2:

Table 3: Kabat numbering of the amino acid residues of the IgG CH2 and CH3 region

IgG subtype	CH2 aa translation	CH2 Kabat numbering	CH3 aa translation	CH3 Kabat numbering
IgG_1	AP*E*… …*KA*K	244… …360	GQP……***P*GK**	361… …478
IgG_2	AP*P*… …*KT*K	244… …360	GQP……***P*GK**	361… …478
IgG_3	AP*E*… …*KT*K	244… …360	GQP……***P*GK**	361… …478
IgG_4	AP*E*… …*KA*K	244… …360	GQP……***L*GK**	361… …478

[113] In one embodiment of the invention the emphasized bold amino acid residues in the CH3 domain of the first or both Fc monomers are deleted.

[114] The peptide linker, by whom the polypeptide monomers ("Fc portion" or "Fc monomer") of the third domain are fused to each other, preferably comprises at least 25 amino acid residues (25, 26, 27, 28, 29, 30 etc.). More preferably, this peptide linker comprises at least 30 amino acid residues (30, 31, 32, 33, 34, 35 etc.). It is also preferred that the linker comprises up to 40 amino acid residues, more preferably up to 35 amino acid residues, most preferably exactly 30 amino acid residues. A preferred embodiment of such peptide linker is characterized by the amino acid sequence Gly-Gly-Gly-Gly-Ser, i.e. Gly_4Ser (SEQ ID NO: 187), or polymers thereof, i.e. $(Gly_4Ser)x$, where x is an integer of 5 or greater (e.g. 6, 7 or 8). Preferably the integer is 6 or 7, more preferably the integer is 6.

[115] In the event that a linker is used to fuse the first domain to the second domain, or the first or second domain to the third domain, this linker is preferably of a length and sequence sufficient to ensure that each of the first and second domains can, independently from one another, retain their differential binding specificities. For peptide linkers which connect the at least two binding domains (or two variable domains) in the antigen-binding molecule of the invention, those peptide linkers are preferred which comprise only a few number of amino acid residues, *e.g.* 12 amino acid residues or less. Thus, peptide linkers of 12, 11, 10, 9, 8, 7, 6 or 5 amino acid residues are preferred. An envisaged peptide linker with less than 5 amino acids comprises 4, 3, 2 or one amino acid(s), wherein Gly-rich linkers are preferred. A preferred embodiment of the peptide linker for a fusion the first and the second domain is depicted in SEQ ID NO:1.

A preferred linker embodiment of the peptide linker for a fusion the second and the third domain is a $(Gly)_4$-linker, respectively G_4-linker.

[116] A particularly preferred "single" amino acid in the context of one of the above described "peptide linker" is Gly. Accordingly, said peptide linker may consist of the single amino acid Gly. In a preferred embodiment of the invention a peptide linker is characterized by the amino acid sequence Gly-Gly-Gly-Gly-Ser, i.e. Gly_4Ser (SEQ ID NO: 187), or polymers thereof, i.e. $(Gly_4Ser)x$, where x is an integer of 1 or greater (e.g. 2 or 3). Preferred linkers are depicted in SEQ ID Nos: 1 to 12. The characteristics of said peptide linker, which comprise the absence of the promotion of secondary structures, are known in the art and are described *e.g.* in Dall'Acqua et al. (Biochem. (1998) 37, 9266-9273), Cheadle et al. (Mol Immunol (1992) 29, 21-30) and Raag and Whitlow (FASEB (1995) 9(1), 73-80). Peptide linkers which furthermore do not promote any secondary structures are preferred. The linkage of said domains to each other can be provided, *e.g.*, by genetic engineering, as described in the examples. Methods for preparing fused and operatively linked bispecific single chain constructs and expressing them in mammalian cells or bacteria are well-known in the art (*e.g.* WO 99/54440 or Sambrook et al., Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, New York, 2001).

[117] In a preferred embodiment of the antigen-binding molecule or the present invention the first and second domain form an antigen-binding molecule in a format selected from the group consisting of $(scFv)_2$, scFv-single domain mAb, diabody and oligomers of any of the those formats

[118] According to a particularly preferred embodiment, and as documented in the appended examples, the first and the second domain of the antigen-binding molecule of the invention is a "bispecific single chain antigen-binding molecule", more preferably a bispecific "single chain Fv" (scFv). Although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker – as described hereinbefore – that enables them to be made as a single protein chain in which the VL and VH regions pair to form a monovalent molecule; see *e.g.*, Huston et al. (1988) Proc. Natl. Acad. Sci USA 85:5879-5883). These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are evaluated for function in the same manner as are whole or full-length antibodies. A single-chain variable fragment (scFv) is hence a fusion protein of the variable region of the heavy chain (VH) and of the light chain (VL) of immunoglobulins, usually connected with a short linker peptide of about ten to about 25 amino acids, preferably about 15 to 20 amino acids. The linker is usually rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the VH with the C-terminus of the VL, or *vice versa*. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and introduction of the linker.

[119] Bispecific single chain antigen-binding molecules are known in the art and are described in WO 99/54440, Mack, J. Immunol. (1997), 158, 3965-3970, Mack, PNAS, (1995), 92, 7021-7025, Kufer,

Cancer Immunol. Immunother., (1997), 45, 193-197, Löffler, Blood, (2000), 95, 6, 2098-2103, Brühl, Immunol., (2001), 166, 2420-2426, Kipriyanov, J. Mol. Biol., (1999), 293, 41-56. Techniques described for the production of single chain antibodies (see, *inter alia*, US Patent 4,946,778, Kontermann and Dübel (2010), *loc. Cit.* and Little (2009), *loc. Cit.*) can be adapted to produce single chain antigen-binding molecules specifically recognizing (an) elected target(s).

[120] Bivalent (also called divalent) or bispecific single-chain variable fragments (bi-scFvs or di-scFvs having the format $(scFv)_2$ can be engineered by linking two scFv molecules (*e.g.* with linkers as described hereinbefore). If these two scFv molecules have the same binding specificity, the resulting $(scFv)_2$ molecule will preferably be called bivalent (*i.e.* it has two valences for the same target epitope). If the two scFv molecules have different binding specificities, the resulting $(scFv)_2$ molecule will preferably be called bispecific. The linking can be done by producing a single peptide chain with two VH regions and two VL regions, yielding tandem scFvs (see *e.g.* Kufer P. *et al.*, (2004) Trends in Biotechnology 22(5):238-244). Another possibility is the creation of scFv molecules with linker peptides that are too short for the two variable regions to fold together (*e.g.* about five amino acids), forcing the scFvs to dimerize. This type is known as diabodies (see *e.g.* Hollinger, Philipp *et al.*, (July 1993) Proceedings of the National Academy of Sciences of the United States of America 90 (14): 6444-8).

[121] In line with this invention either the first, the second or the first and the second domain may comprise a single domain antibody, respectively the variable domain or at least the CDRs of a single domain antibody. Single domain antibodies comprise merely one (monomeric) antibody variable domain which is able to bind selectively to a specific antigen, independently of other V regions or domains. The first single domain antibodies were engineered from havy chain antibodies found in camelids, and these are called V_HH fragments. Cartilaginous fishes also have heavy chain antibodies (IgNAR) from which single domain antibodies called V_{NAR} fragments can be obtained. An alternative approach is to split the dimeric variable domains from common immunoglobulins *e.g.* from humans or rodents into monomers, hence obtaining VH or VL as a single domain Ab. Although most research into single domain antibodies is currently based on heavy chain variable domains, nanobodies derived from light chains have also been shown to bind specifically to target epitopes. Examples of single domain antibodies are called sdAb, nanobodies or single variable domain antibodies.

[122] A $(\text{single domain mAb})_2$ is hence a monoclonal antigen-binding molecule composed of (at least) two single domain monoclonal antibodies, which are individually selected from the group comprising V_H, V_L, V_HH and V_{NAR}. The linker is preferably in the form of a peptide linker. Similarly, an "scFv-single domain mAb" is a monoclonal antigen-binding molecule composed of at least one single domain antibody as described above and one scFv molecule as described above. Again, the linker is preferably in the form of a peptide linker.

[123] Whether or not an antigen-binding molecule competes for binding with another given antigen-binding molecule can be measured in a competition assay such as a competitive ELISA or a cell-based competition assay. Avidin-coupled microparticles (beads) can also be used. Similar to an avidin-coated ELISA plate, when reacted with a biotinylated protein, each of these beads can be used as a substrate on which an assay can be performed. Antigen is coated onto a bead and then precoated with the first antibody. The second antibody is added and any additional binding is determined. Possible means for the read-out includes flow cytometry.

[124] T cells or T lymphocytes are a type of lymphocyte (itself a type of white blood cell) that play a central role in cell-mediated immunity. There are several subsets of T cells, each with a distinct function. T cells can be distinguished from other lymphocytes, such as B cells and NK cells, by the presence of a T cell receptor (TCR) on the cell surface. The TCR is responsible for recognizing antigens bound to major histocompatibility complex (MHC) molecules and is composed of two different protein chains. In 95% of the T cells, the TCR consists of an alpha (α) and beta (β) chain. When the TCR engages with antigenic peptide and MHC (peptide / MHC complex), the T lymphocyte is activated through a series of biochemical events mediated by associated enzymes, co-receptors, specialized adaptor molecules, and activated or released transcription factors.

[125] The CD3 receptor complex is a protein complex and is composed of four chains. In mammals, the complex contains a CD3γ (gamma) chain, a CD3δ (delta) chain, and two CD3ε (epsilon) chains. These chains associate with the T cell receptor (TCR) and the so-called ζ (zeta) chain to form the T cell receptor CD3 complex and to generate an activation signal in T lymphocytes. The CD3γ (gamma), CD3δ (delta), and CD3ε (epsilon) chains are highly related cell-surface proteins of the immunoglobulin superfamily containing a single extracellular immunoglobulin domain. The intracellular tails of the CD3 molecules contain a single conserved motif known as an immunoreceptor tyrosine-based activation motif or ITAM for short, which is essential for the signaling capacity of the TCR. The CD3 epsilon molecule is a polypeptide which in humans is encoded by the *CD3E* gene which resides on chromosome 11. The most preferred epitope of CD3 epsilon is comprised within amino acid residues 1-27 of the human CD3 epsilon extracellular domain. It is envisaged that antigen-binding molecules according to the present invention typically and advantageously show less unspecific T cell activation, which is not desired in specific immunotherapy. This translates to a reduced risk of side effects.

[126] The redirected lysis of target cells via the recruitment of T cells by a multispecific, at least bispecific, antigen-binding molecule involves cytolytic synapse formation and delivery of perforin and granzymes. The engaged T cells are capable of serial target cell lysis, and are not affected by immune escape mechanisms interfering with peptide antigen processing and presentation, or clonal T cell differentiation; see, for example, WO 2007/042261.

[127] Cytotoxicity mediated by antigen-binding molecules of the invention can be measured in various ways. Effector cells can be e.g. stimulated enriched (human) CD8 positive T cells or unstimulated (human) peripheral blood mononuclear cells (PBMC). If the target cells are of macaque origin or express or are transfected with macaque target cell surface antigen which is bound by the first domain, the effector cells should also be of macaque origin such as a macaque T cell line, e.g. 4119LnPx. The target cells should express (at least the extracellular domain of) the target cell surface antigen, e.g. human or macaque target cell surface antigen. Target cells can be a cell line (such as CHO) which is stably or transiently transfected with target cell surface antigen, e.g. human or macaque target cell surface antigen. Alternatively, the target cells can be a target cell surface antigen positive natural expresser cell line. Usually EC_{50} values are expected to be lower with target cell lines expressing higher levels of target cell surface antigen on the cell surface. The effector to target cell (E:T) ratio is usually about 10:1, but can also vary. Cytotoxic activity of target cell surface antigenxCD3 bispecific antigen-binding molecules can be measured in a ^{51}Cr-release assay (incubation time of about 18 hours) or in a in a FACS-based cytotoxicity assay (incubation time of about 48 hours). Modifications of the assay incubation time (cytotoxic reaction) are also possible. Other methods of measuring cytotoxicity are well-known to the skilled person and comprise MTT or MTS assays, ATP-based assays including bioluminescent assays, the sulforhodamine B (SRB) assay, WST assay, clonogenic assay and the ECIS technology.

[128] The cytotoxic activity mediated by target cell surface antigenxCD3 bispecific antigen-binding molecules of the present invention is preferably measured in a cell-based cytotoxicity assay. It may also be measured in a ^{51}Cr-release assay. It is represented by the EC_{50} value, which corresponds to the half maximal effective concentration (concentration of the antigen-binding molecule which induces a cytotoxic response halfway between the baseline and maximum). Preferably, the EC_{50} value of the target cell surface antigenxCD3 bispecific antigen-binding molecules is $\leq$5000 pM or $\leq$4000 pM, more preferably $\leq$3000 pM or $\leq$2000 pM, even more preferably $\leq$1000 pM or $\leq$500 pM, even more preferably $\leq$400 pM or $\leq$300 pM, even more preferably $\leq$200 pM, even more preferably $\leq$100 pM, even more preferably $\leq$50 pM, even more preferably $\leq$20 pM or $\leq$10 pM, and most preferably $\leq$5 pM.

[129] The above given EC_{50} values can be measured in different assays. The skilled person is aware that an EC_{50} value can be expected to be lower when stimulated / enriched $CD8^+$ T cells are used as effector cells, compared with unstimulated PBMC. It can furthermore be expected that the EC_{50} values are lower when the target cells express a high number of the target cell surface antigen compared with a low target expression rat. For example, when stimulated / enriched human $CD8^+$ T cells are used as effector cells (and either target cell surface antigen transfected cells such as CHO cells or target cell surface antigen positive human cell lines are used as target cells), the EC_{50} value of the target cell surface antigenxCD3 bispecific antigen-binding molecule is preferably $\leq$1000 pM, more preferably $\leq$500 pM, even more preferably $\leq$250 pM, even more preferably $\leq$100 pM, even more preferably $\leq$50 pM, even more preferably $\leq$10 pM, and most preferably $\leq$5 pM. When human PBMCs are used as effector cells, the EC_{50} value of the target

cell surface antigenxCD3 bispecific antigen-binding molecule is preferably ≤5000 pM or ≤4000 pM (in particular when the target cells are target cell surface antigen positive human cell lines), more preferably ≤2000 pM (in particular when the target cells are target cell surface antigen transfected cells such as CHO cells), more preferably ≤1000 pM or ≤500 pM, even more preferably ≤200 pM, even more preferably ≤150 pM, even more preferably ≤100 pM, and most preferably ≤50 pM, or lower. When a macaque T cell line such as LnPx4119 is used as effector cells, and a macaque target cell surface antigen transfected cell line such as CHO cells is used as target cell line, the EC_{50} value of the target cell surface antigenxCD3 bispecific antigen-binding molecule is preferably ≤2000 pM or ≤1500 pM, more preferably ≤1000 pM or ≤500 pM, even more preferably ≤300 pM or ≤250 pM, even more preferably ≤100 pM, and most preferably ≤50 pM.

[130] Preferably, the target cell surface antigenxCD3 bispecific antigen-binding molecules of the present invention do not induce / mediate lysis or do not essentially induce / mediate lysis of target cell surface antigen negative cells such as CHO cells. The term "do not induce lysis", "do not essentially induce lysis", "do not mediate lysis" or "do not essentially mediate lysis" means that an antigen-binding molecule of the present invention does not induce or mediate lysis of more than 30%, preferably not more than 20%, more preferably not more than 10%, particularly preferably not more than 9%, 8%, 7%, 6% or 5% of target cell surface antigen negative cells, whereby lysis of a target cell surface antigen positive human cell line is set to be 100%. This usually applies for concentrations of the antigen-binding molecule of up to 500 nM. The skilled person knows how to measure cell lysis without further ado. Moreover, the present specification teaches specific instructions how to measure cell lysis.

[131] The difference in cytotoxic activity between the monomeric and the dimeric isoform of individual target cell surface antigenxCD3 bispecific antigen-binding molecules is referred to as "potency gap". This potency gap can *e.g.* be calculated as ratio between EC_{50} values of the molecule's monomeric and dimeric form. Potency gaps of the target cell surface antigenxCD3 bispecific antigen-binding molecules of the present invention are preferably ≤ 5, more preferably ≤ 4, even more preferably ≤ 3, even more preferably ≤ 2 and most preferably ≤ 1.

[132] The first and/or the second (or any further) binding domain(s) of the antigen-binding molecule of the invention is/are preferably cross-species specific for members of the mammalian order of primates. Cross-species specific CD3 binding domains are, for example, described in WO 2008/119567. According to one embodiment, the first and/or second binding domain, in addition to binding to human target cell surface antigen and human CD3, respectively, will also bind to target cell surface antigen / CD3 of primates including (but not limited to) new world primates (such as *Callithrix jacchus*, *Saguinus Oedipus* or *Saimiri sciureus*), old world primates (such baboons and macaques), gibbons, and non-human *hominidae*.

[133] In one embodiment of the antigen-binding molecule of the invention the first domain binds to human target cell surface antigen and further binds to macaque target cell surface antigen, such as target

cell surface antigen of *Macaca fascicularis*, and more preferably, to macaque target cell surface antigen expressed on the surface macaque cells. The affinity of the first binding domain for macaque target cell surface antigen is preferably ≤15 nM, more preferably ≤10 nM, even more preferably ≤5 nM, even more preferably ≤1 nM, even more preferably ≤0.5 nM, even more preferably ≤0.1 nM, and most preferably ≤0.05 nM or even ≤0.01 nM.

[134] Preferably the affinity gap of the antigen-binding molecules according to the invention for binding macaque target cell surface antigen versus human target cell surface antigen [ma target cell surface antigen:hu target cell surface antigen] (as determined *e.g.* by BiaCore or by Scatchard analysis) is <100, preferably <20, more preferably <15, further preferably <10, even more preferably<8, more preferably <6 and most preferably <2. Preferred ranges for the affinity gap of the antigen-binding molecules according to the invention for binding macaque target cell surface antigen versus human target cell surface antigen are between 0.1 and 20, more preferably between 0.2 and 10, even more preferably between 0.3 and 6, even more preferably between 0.5 and 3 or between 0.5 and 2.5, and most preferably between 0.5 and 2 or between 0.6 and 2.

[135] The second (binding) domain of the antigen-binding molecule of the invention binds to human CD3 epsilon and/or to *Macaca* CD3 epsilon. In a preferred embodiment the second domain further bind to *Callithrix jacchus*, *Saguinus Oedipus* or *Saimiri sciureus* CD3 epsilon. *Callithrix jacchus and Saguinus oedipus* are both new world primate belonging to the family of *Callitrichidae*, while *Saimiri sciureus* is a new world primate belonging to the family of *Cebidae*.

[136] It is preferred for the antigen-binding molecule of the present invention that the second domain which binds to an extracellular epitope of the human and/or the *Macaca* CD3 on the comprises a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from:

(a) CDR-L1 as depicted in SEQ ID NO: 27 of WO 2008/119567, CDR-L2 as depicted in SEQ ID NO: 28 of WO 2008/119567 and CDR-L3 as depicted in SEQ ID NO: 29 of WO 2008/119567;

(b) CDR-L1 as depicted in SEQ ID NO: 117 of WO 2008/119567, CDR-L2 as depicted in SEQ ID NO: 118 of WO 2008/119567 and CDR-L3 as depicted in SEQ ID NO: 119 of WO 2008/119567; and

I CDR-L1 as depicted in SEQ ID NO: 153 of WO 2008/119567, CDR-L2 as depicted in SEQ ID NO: 154 of WO 2008/119567 and CDR-L3 as depicted in SEQ ID NO: 155 of WO 2008/119567.

[137] In an also preferred embodiment of the antigen-binding molecule of the present invention, the second domain which binds to an extracellular epitope of the human and/or the *Macaca* CD3 epsilon chain comprises a VH region comprising CDR-H 1, CDR-H2 and CDR-H3 selected from:

(a) CDR-H1 as depicted in SEQ ID NO: 12 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 13 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 14 of WO 2008/119567;

(b) CDR-H1 as depicted in SEQ ID NO: 30 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 31 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 32 of WO 2008/119567;

I CDR-H1 as depicted in SEQ ID NO: 48 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 49 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 50 of WO 2008/119567;

(d) CDR-H1 as depicted in SEQ ID NO: 66 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 67 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 68 of WO 2008/119567;

I CDR-H1 as depicted in SEQ ID NO: 84 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 85 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 86 of WO 2008/119567;

(f) CDR-H1 as depicted in SEQ ID NO: 102 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 103 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 104 of WO 2008/119567;

(g) CDR-H1 as depicted in SEQ ID NO: 120 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 121 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 122 of WO 2008/119567;

(h) CDR-H1 as depicted in SEQ ID NO: 138 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 139 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 140 of WO 2008/119567;

(i) CDR-H1 as depicted in SEQ ID NO: 156 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 157 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 158 of WO 2008/119567; and

(j) CDR-H1 as depicted in SEQ ID NO: 174 of WO 2008/119567, CDR-H2 as depicted in SEQ ID NO: 175 of WO 2008/119567 and CDR-H3 as depicted in SEQ ID NO: 176 of WO 2008/119567.

[138] In a preferred embodiment of the antigen-binding molecule of the invention the above described three groups of VL CDRs are combined with the above described ten groups of VH CDRs within the second binding domain to form (30) groups, each comprising CDR-L 1-3 and CDR-H 1-3.

[139] It is preferred for the antigen-binding molecule of the present invention that the second domain which binds to CD3 comprises a VL region selected from the group consisting of a VL region as depicted in SEQ ID NO: 17, 21, 35, 39, 53, 57, 71, 75, 89, 93, 107, 111, 125, 129, 143, 147, 161, 165, 179 or 183 of WO 2008/119567 or as depicted in SEQ ID NO: 200.

[140] It is also preferred that the second domain which binds to CD3 comprises a VH region selected from the group consisting of a VH region as depicted in SEQ ID NO: 15, 19, 33, 37, 51, 55, 69, 73, 87, 91, 105, 109, 123, 127, 141, 145, 159, 163, 177 or 181 of WO 2008/119567 or as depicted in SEQ ID NO: 201.

[141] More preferably, the antigen-binding molecule of the present invention is characterized by a second domain which binds to CD3 comprising a VL region and a VH region selected from the group consisting of:

(a) a VL region as depicted in SEQ ID NO: 17 or 21 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 15 or 19 of WO 2008/119567;

(b) a VL region as depicted in SEQ ID NO: 35 or 39 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 33 or 37 of WO 2008/119567;

I a VL region as depicted in SEQ ID NO: 53 or 57 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 51 or 55 of WO 2008/119567;

(d) a VL region as depicted in SEQ ID NO: 71 or 75 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 69 or 73 of WO 2008/119567;

I a VL region as depicted in SEQ ID NO: 89 or 93 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 87 or 91 of WO 2008/119567;

(f) a VL region as depicted in SEQ ID NO: 107 or 111 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 105 or 109 of WO 2008/119567;

(g) a VL region as depicted in SEQ ID NO: 125 or 129 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 123 or 127 of WO 2008/119567;

(h) a VL region as depicted in SEQ ID NO: 143 or 147 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 141 or 145 of WO 2008/119567;

(i) a VL region as depicted in SEQ ID NO: 161 or 165 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 159 or 163 of WO 2008/119567;

(j) a VL region as depicted in SEQ ID NO: 179 or 183 of WO 2008/119567 and a VH region as depicted in SEQ ID NO: 177 or 181 of WO 2008/119567; and

(k) a VL region as depicted in SEQ ID NO: 60 of WO 2022/096716 and a VH region as depicted in SEQ ID NO: 59 of WO 2022/096716.

[142] Also preferred in connection with the antigen-binding molecule of the present invention is a second domain which binds to CD3 comprising a VL region as depicted in SEQ ID NO: 200 and a VH region as depicted in SEQ ID NO: 201.

[143] According to a preferred embodiment of the antigen-binding molecule of the present invention, the first and/or the second domain have the following format: The pairs of VH regions and VL regions are in the format of a single chain antibody (scFv). The VH and VL regions are arranged in the order VH-VL or VL-VH. It is preferred that the VH-region is positioned N-terminally of a linker sequence, and the VL-region is positioned C-terminally of the linker sequence.

[144] A preferred embodiment of the above described antigen-binding molecule of the present invention is characterized by the second domain which binds to CD3 comprising an amino acid sequence selected from the group consisting of SEQ ID Nos: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187 of WO 2008/119567, or depicted in SEQ ID NO: 202 (named herein I2C), or the second domain in N- to C-terminal order consisting of (i.) VH region as depicted in SEQ ID NO: 59 of WO 2022/096716, (ii.) SEQ ID NO 189, and (iii.) VL region as depicted in SEQ ID NO: 60 of WO 2022/096716 (named herein I2E). As discussed herein, without wanting to be bound be theory, the stabilizing agent according to the invention typically interacts with and stabilizes the second, i.e. CD3 binding domain despite structural differences between examples of said second domain, e.g. between I2C and I2E.

[145] Covalent modifications of the antigen-binding molecules are also included within the scope of this invention, and are generally, but not always, done post-translationally. For example, several types of covalent modifications of the antigen-binding molecule are introduced into the molecule by reacting specific amino acid residues of the antigen-binding molecule with an organic derivatizing agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

[146] Cysteinyl residues most commonly are reacted with α-haloacetates (and corresponding amines), such as chloroacetic acid or chloroacetamide, to give carboxymethyl or carboxyamidomethyl derivatives. Cysteinyl residues also are derivatized by reaction with bromotrifluoroacetone, α-bromo-β-(5-imidozoyl)propionic acid, chloroacetyl phosphate, N-alkylmaleimides, 3-nitro-2-pyridyl disulfide, methyl 2-pyridyl disulfide, p-chloromercuribenzoate, 2-chloromercuri-4-nitrophenol, or chloro-7-nitrobenzo-2-oxa-1,3-diazole.

[147] Histidyl residues are derivatized by reaction with diethylpyrocarbonate at pH 5.5-7.0 because this agent is relatively specific for the histidyl side chain. Para-bromophenacyl bromide also is useful; the reaction is preferably performed in 0.1 M sodium cacodylate at pH 6.0. Lysinyl and amino terminal residues are reacted with succinic or other carboxylic acid anhydrides. Derivatization with these agents has the effect of reversing the charge of the lysinyl residues. Other suitable reagents for derivatizing alpha-amino-containing residues include imidoesters such as methyl picolinimidate; pyridoxal phosphate; pyridoxal; chloroborohydride; trinitrobenzenesulfonic acid; O-methylisourea; 2,4-pentanedione; and transaminase-catalyzed reaction with glyoxylate.

[148] Arginyl residues are modified by reaction with one or several conventional reagents, among them phenylglyoxal, 2,3-butanedione, 1,2-cyclohexanedione, and ninhydrin. Derivatization of arginine residues requires that the reaction be performed in alkaline conditions because of the high pKa of the guanidine functional group. Furthermore, these reagents may react with the groups of lysine as well as the arginine epsilon-amino group.

[149] The specific modification of tyrosyl residues may be made, with particular interest in introducing spectral labels into tyrosyl residues by reaction with aromatic diazonium compounds or tetranitromethane. Most commonly, N-acetylimidizole and tetranitromethane are used to form O-acetyl tyrosyl species and 3-nitro derivatives, respectively. Tyrosyl residues are iodinated using ^{125}I or ^{131}I to prepare labeled proteins for use in radioimmunoassay, the chloramine T method described above being suitable.

[150] Carboxyl side groups (aspartyl or glutamyl) are selectively modified by reaction with carbodiimides (R'—N=C=N–R'), where R and R' are optionally different alkyl groups, such as 1-cyclohexyl-3-(2-morpholinyl-4-ethyl) carbodiimide or 1-ethyl-3-(4-azonia-4,4-dimethylpentyl) carbodiimide. Furthermore, aspartyl and glutamyl residues are converted to asparaginyl and glutaminyl residues by reaction with ammonium ions.

[151] Derivatization with bifunctional agents is useful for crosslinking the antigen-binding molecules of the present invention to a water-insoluble support matrix or surface for use in a variety of methods. Commonly used crosslinking agents include, *e.g.*, 1,1-bis(diazoacetyl)-2-phenylethane, glutaraldehyde, N-hydroxysuccinimide esters, for example, esters with 4-azidosalicylic acid, homobifunctional imidoesters, including disuccinimidyl esters such as 3,3'-dithiobis(succinimidylpropionate), and bifunctional maleimides such as bis-N-maleimido-1,8-octane. Derivatizing agents such as methyl-3-[(p-azidophenyl)dithio]propioimidate yield photoactivatable intermediates that are capable of forming crosslinks in the presence of light. Alternatively, reactive water-insoluble matrices such as cyanogen bromide-activated carbohydrates and the reactive substrates as described in U.S. Pat. Nos. 3,969,287; 3,691,016; 4,195,128; 4,247,642; 4,229,537; and 4,330,440 are employed for protein immobilization.

[152] Glutaminyl and asparaginyl residues are frequently deamidated to the corresponding glutamyl and aspartyl residues, respectively. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues falls within the scope of this invention.

[153] Other modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the α-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, Proteins: Structure and Molecular Properties, W. H. Freeman & Co., San Francisco, 1983, pp. 79-86), acetylation of the N-terminal amine, and amidation of any C-terminal carboxyl group.

[154] Another type of covalent modification of the antigen-binding molecules included within the scope of this invention comprises altering the glycosylation pattern of the protein. As is known in the art, glycosylation patterns can depend on both the sequence of the protein (*e.g.*, the presence or absence of particular glycosylation amino acid residues, discussed below), or the host cell or organism in which the protein is produced. Particular expression systems are discussed below.

[155] Glycosylation of polypeptides is typically either N-linked or O-linked. N-linked refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tri-peptide sequences asparagine-X-serine and asparagine-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tri-peptide sequences in a polypeptide creates a potential glycosylation site. O-linked glycosylation refers to the attachment of one of the sugars N-acetylgalactosamine, galactose, or xylose, to a hydroxyamino acid, most commonly serine or threonine, although 5-hydroxyproline or 5-hydroxylysine may also be used.

[156] Addition of glycosylation sites to the antigen-binding molecule is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tri-peptide sequences (for N-linked glycosylation sites). The alteration may also be made by the addition of, or

substitution by, one or more serine or threonine residues to the starting sequence (for O-linked glycosylation sites). For ease, the amino acid sequence of an antigen-binding molecule is preferably altered through changes at the DNA level, particularly by mutating the DNA encoding the polypeptide at preselected bases such that codons are generated that will translate into the desired amino acids.

[157] Another means of increasing the number of carbohydrate moieties on the antigen-binding molecule is by chemical or enzymatic coupling of glycosides to the protein. These procedures are advantageous in that they do not require production of the protein in a host cell that has glycosylation capabilities for N- and O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as those of cysteine, (d) free hydroxyl groups such as those of serine, threonine, or hydroxyproline, I aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. These methods are described in WO 87/05330, and in Aplin and Wriston, 1981, CRC Crit. Rev. Biochem., pp. 259-306.

[158] Removal of carbohydrate moieties present on the starting antigen-binding molecule may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the protein to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the polypeptide intact. Chemical deglycosylation is described by Hakimuddin *et al.*, 1987, *Arch. Biochem. Biophys.* 259:52 and by Edge *et al.*, 1981, *Anal. Biochem.* 118:131. Enzymatic cleavage of carbohydrate moieties on polypeptides can be achieved by the use of a variety of endo- and exo-glycosidases as described by Thotakura *et al.*, 1987, Meth. Enzymol. 138:350. Glycosylation at potential glycosylation sites may be prevented by the use of the compound tunicamycin as described by Duskin *et al.*, 1982, J. Biol. Chem. 257:3105. Tunicamycin blocks the formation of protein-N-glycoside linkages.

[159] Other modifications of the antigen-binding molecule are also contemplated herein. For example, another type of covalent modification of the antigen-binding molecule comprises linking the antigen-binding molecule to various non-proteinaceous polymers, including, but not limited to, various polyols such as polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol, in the manner set forth in U.S. Patent Nos. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337. In addition, as is known in the art, amino acid substitutions may be made in various positions within the antigen-binding molecule, e.g. in order to facilitate the addition of polymers such as PEG.

[160] In some embodiments, the covalent modification of the antigen-binding molecules of the invention comprises the addition of one or more labels. The labelling group may be coupled to the antigen-binding molecule *via* spacer arms of various lengths to reduce potential steric hindrance. Various methods for labelling proteins are known in the art and can be used in performing the present invention. The term "label" or "labelling group" refers to any detectable label. In general, labels fall into a variety of classes,

depending on the assay in which they are to be detected – the following examples include, but are not limited to:

a) isotopic labels, which may be radioactive or heavy isotopes, such as radioisotopes or radionuclides (e.g., ^{3}H, ^{14}C, ^{15}N, ^{35}S, ^{89}Zr, ^{90}Y, ^{99}Tc, ^{111}In, ^{125}I, ^{131}I)

b) magnetic labels (e.g., magnetic particles)

c) redox active moieties

d) optical dyes (including, but not limited to, chromophores, phosphors and fluorophores) such as fluorescent groups (e.g., FITC, rhodamine, lanthanide phosphors), chemiluminescent groups, and fluorophores which can be either "small molecule" fluores or proteinaceous fluores

e) enzymatic groups (e.g. horseradish peroxidase, β-galactosidase, luciferase, alkaline phosphatase)

f) biotinylated groups

g) predetermined polypeptide epitopes recognized by a secondary reporter (*e.g.*, leucine zipper pair sequences, binding sides for secondary antibodies, metal binding domains, epitope tags, etc.)

[161] By "fluorescent label" is meant any molecule that may be detected *via* its inherent fluorescent properties. Suitable fluorescent labels include, but are not limited to, fluorescein, rhodamine, tetramethylrhodamine, eosin, erythrosin, coumarin, methyl-coumarins, pyrene, Malacite green, stilbene, Lucifer Yellow, Cascade BlueJ, Texas Red, IAEDANS, EDANS, BODIPY FL, LC Red 640, Cy 5, Cy 5.5, LC Red 705, Oregon green, the Alexa-Fluor dyes (Alexa Fluor 350, Alexa Fluor 430, Alexa Fluor 488, Alexa Fluor 546, Alexa Fluor 568, Alexa Fluor 594, Alexa Fluor 633, Alexa Fluor 660, Alexa Fluor 680), Cascade Blue, Cascade Yellow and R-phycoerythrin (PE) (Molecular Probes, Eugene, OR), FITC, Rhodamine, and Texas Red (Pierce, Rockford, IL), Cy5, Cy5.5, Cy7 (Amersham Life Science, Pittsburgh, PA). Suitable optical dyes, including fluorophores, are described in Molecular Probes Handbook by Richard P. Haugland.

[162] Suitable proteinaceous fluorescent labels also include, but are not limited to, green fluorescent protein, including a Renilla, Ptilosarcus, or Aequorea species of GFP (Chalfie *et al.*, 1994, *Science* 263:802-805), EGFP (Clontech Laboratories, Inc., Genbank Accession Number U55762), blue fluorescent protein (BFP, Quantum Biotechnologies, Inc. 1801 de Maisonneuve Blvd. West, 8th Floor, Montreal, Quebec, Canada H3H 1J9; Stauber, 1998, *Biotechniques* 24:462-471; Heim *et al.*, 1996, *Curr. Biol.* 6:178-182), enhanced yellow fluorescent protein (EYFP, Clontech Laboratories, Inc.), luciferase (Ichiki *et al.*, 1993, *J. Immunol.* 150:5408-5417), β galactosidase (Nolan *et al.*, 1988, *Proc. Natl. Acad. Sci. U.S.A.* 85:2603-2607) and Renilla (WO92/15673, WO95/07463, WO98/14605, WO98/26277, WO99/49019, U.S. Patent Nos. 5,292,658; 5,418,155; 5,683,888; 5,741,668; 5,777,079; 5,804,387; 5,874,304; 5,876,995; 5,925,558).

[163] The antigen-binding molecule of the invention may also comprise additional domains, which are *e.g.* helpful in the isolation of the molecule or relate to an adapted pharmacokinetic profile of the molecule. Domains helpful for the isolation of an antigen-binding molecule may be selected from peptide motives or

secondarily introduced moieties, which can be captured in an isolation method, e.g. an isolation column. Non-limiting embodiments of such additional domains comprise peptide motives known as Myc-tag, HAT-tag, HA-tag, TAP-tag, GST-tag, chitin binding domain (CBD-tag), maltose binding protein (MBP-tag), Flag-tag, Strep-tag and variants thereof (e.g. StrepII-tag) and His-tag. All herein disclosed antigen-binding molecules characterized by the identified CDRs may comprise a His-tag domain, which is generally known as a repeat of consecutive His residues in the amino acid sequence of a molecule, preferably of five, and more preferably of six His residues (hexa-histidine). The His-tag may be located *e.g.* at the N- or C-terminus of the antigen-binding molecule, preferably it is located at the C-terminus. Most preferably, a hexa-histidine tag (HHHHHH) (SEQ ID NO:199) is linked via peptide bond to the C-terminus of the antigen-binding molecule according to the invention. Additionally, a conjugate system of PLGA-PEG-PLGA may be combined with a poly-histidine tag for sustained release application and improved pharmacokinetic profile.

[164] Amino acid sequence modifications of the antigen-binding molecules described herein are also contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antigen-binding molecule. Amino acid sequence variants of the antigen-binding molecules are prepared by introducing appropriate nucleotide changes into the antigen-binding molecules nucleic acid, or by peptide synthesis. All of the below described amino acd sequence modifications should result in an antigen-binding molecule which still retains the desired biological activity (binding to the target cell surface antigen and to CD3) of the unmodified parental molecule.

[165] The term “amino acid” or “amino acid residue” typically refers to an amino acid having its art recognized definition such as an amino acid selected from the group consisting of: alanine (Ala or A); arginine (Arg or R); asparagine (Asn or N); aspartic acid (Asp or D); cysteine (Cys or C); glutamine (Gin or Q); glutamic acid (Giu or E); glycine (Giy or G); histidine (His or H); isoleucine (He or I): leucine (Leu or L); lysine (Lys or K); methionine (Met or M); phenylalanine (Phe or F); pro line (Pro or P); serine (Ser or S); threonine (Thr or T); tryptophan (Trp or W); tyrosine (Tyr or Y); and valine (VaI or V), although modified, synthetic, or rare amino acids may be used as desired. Generally, amino acids can be grouped as having a nonpolar side chain (e.g., Ala, Cys, He, Leu, Met, Phe, Pro, VaI); a negatively charged side chain (e.g., Asp, Giu); a positively charged sidechain (e.g., Arg, His, Lys); or an uncharged polar side chain (e.g., Asn, Cys, Gin, Giy, His, Met, Phe, Ser, Thr, Trp, and Tyr).

[166] Amino acid modifications include, for example, deletions from, and/or insertions into, and/or substitutions of, residues within the amino acid sequences of the antigen-binding molecules. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the antigen-binding molecules, such as changing the number or position of glycosylation sites.

[167] For example, 1, 2, 3, 4, 5, or 6 amino acids may be inserted, substituted or deleted in each of the CDRs (of course, dependent on their length), while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be inserted, substituted or deleted in each of the FRs. Preferably, amino acid sequence insertions into the antigen-binding molecule include amino- and/or carboxyl-terminal fusions ranging in length from 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 residues to polypeptides containing a hundred or more residues, as well as intra-sequence insertions of single or multiple amino acid residues. Corresponding modifications may also performed within the third domain of the antigen-binding molecule of the invention. An insertional variant of the antigen-binding molecule of the invention includes the fusion to the N-terminus or to the C-terminus of the antigen-binding molecule of an enzyme or the fusion to a polypeptide.

[168] The sites of greatest interest for substitutional mutagenesis include (but are not limited to) the CDRs of the heavy and/or light chain, in particular the hypervariable regions, but FR alterations in the heavy and/or light chain are also contemplated. The substitutions are preferably conservative substitutions as described herein. Preferably, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids may be substituted in a CDR, while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be substituted in the framework regions (FRs), depending on the length of the CDR or FR. For example, if a CDR sequence encompasses 6 amino acids, it is envisaged that one, two or three of these amino acids are substituted. Similarly, if a CDR sequence encompasses 15 amino acids it is envisaged that one, two, three, four, five or six of these amino acids are substituted.

[169] A useful method for identification of certain residues or regions of the antigen-binding molecules that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells in Science, 244: 1081-1085 (1989). Here, a residue or group of target residues within the antigen-binding molecule is/are identified (*e.g.* charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with the epitope.

[170] Those amino acid locations demonstrating functional sensitivity to the substitutions are then refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site or region for introducing an amino acid sequence variation is predetermined, the nature of the mutation *per se* needs not to be predetermined. For example, to analyze or optimize the performance of a mutation at a given site, alanine scanning or random mutagenesis may be conducted at a target codon or region, and the expressed antigen-binding molecule variants are screened for the optimal combination of desired activity. Techniques for making substitution mutations at predetermined sites in the DNA having a known sequence are well known, for example, M13 primer mutagenesis and PCR mutagenesis. Screening of the mutants is done using assays of antigen binding activities, such as the target cell surface antigen or CD3 binding.

[171] Generally, if amino acids are substituted in one or more or all of the CDRs of the heavy and/or light chain, it is preferred that the then-obtained "substituted" sequence is at least 60% or 65%, more preferably 70% or 75%, even more preferably 80% or 85%, and particularly preferably 90% or 95% identical to the "original" CDR sequence. This means that it is dependent of the length of the CDR to which degree it is identical to the "substituted" sequence. For example, a CDR having 5 amino acids is preferably 80% identical to its substituted sequence in order to have at least one amino acid substituted. Accordingly, the CDRs of the antigen-binding molecule may have different degrees of identity to their substituted sequences, e.g., CDRL1 may have 80%, while CDRL3 may have 90%.

[172] Preferred substitutions (or replacements) are conservative substitutions. However, any substitution (including non-conservative substitution or one or more from the "exemplary substitutions" listed in Table 3, below) is envisaged as long as the antigen-binding molecule retains its capability to bind to the target cell surface antigen via the first domain and to CD3, respectively CD3 epsilon, via the second domain and/or its CDRs have an identity to the then substituted sequence (at least 60% or 65%, more preferably 70% or 75%, even more preferably 80% or 85%, and particularly preferably 90% or 95% identical to the "original" CDR sequence).

[173] Conservative substitutions are shown in Table 3 under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 3, or as further described below in reference to amino acid classes, may be introduced and the products screened for a desired characteristic.

Table 4: Amino acid substitutions

Original	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val, leu, ile	Val
Arg I	lys, gln, asn	Lys
Asn (N)	gln, his, asp, lys, arg	Gln
Asp (D)	glu, asn	Glu
Cys I	ser, ala	Ser
Gln (Q)	asn, glu	Asn
Glu I	asp, gln	Asp
Gly (G)	Ala	Ala
His (H)	asn, gln, lys, arg	Arg
Ile (I)	leu, val, met, ala, phe	Leu
Leu (L)	norleucine, ile, val, met, ala	Ile
Lys (K)	arg, gln, asn	Arg
Met (M)	leu, phe, ile	Leu

Phe (F)	leu, val, ile, ala, tyr	Tyr
Pro (P)	Ala	Ala
Ser (S)	Thr	Thr
Thr (T)	Ser	Ser
Trp (W)	tyr, phe	Tyr
Tyr (Y)	trp, phe, thr, ser	Phe
Val (V)	ile, leu, met, phe, ala	Leu

[174] Substantial modifications in the biological properties of the antigen-binding molecule of the present invention are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties: (1) hydrophobic: norleucine, met, ala, val, leu, ile; (2) neutral hydrophilic: cys, ser, thr, asn, gln; (3) acidic: asp, glu; (4) basic: his, lys, arg; (5) residues that influence chain orientation: gly, pro; and (6) aromatic : trp, tyr, phe.

[175] Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Any cysteine residue not involved in maintaining the proper conformation of the antigen-binding molecule may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking. Conversely, cysteine bond(s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[176] For amino acid sequences, sequence identity and/or similarity is determined by using standard techniques known in the art, including, but not limited to, the local sequence identity algorithm of Smith and Waterman, 1981, *Adv. Appl. Math.* 2:482, the sequence identity alignment algorithm of Needleman and Wunsch, 1970, *J. Mol. Biol.* 48:443, the search for similarity method of Pearson and Lipman, 1988, *Proc. Nat. Acad. Sci. U.S.A.* 85:2444, computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.), the Best Fit sequence program described by Devereux *et al.*, 1984, *Nucl. Acid Res.* 12:387-395, preferably using the default settings, or by inspection. Preferably, percent identity is calculated by FastDB based upon the following parameters: mismatch penalty of 1; gap penalty of 1; gap size penalty of 0.33; and joining penalty of 30, "Current Methods in Sequence Comparison and Analysis," Macromolecule Sequencing and Synthesis, Selected Methods and Applications, pp 127-149 (1988), Alan R. Liss, Inc.

[177] An example of a useful algorithm is PILEUP. PILEUP creates a multiple sequence alignment from a group of related sequences using progressive, pairwise alignments. It can also plot a tree showing the

clustering relationships used to create the alignment. PILEUP uses a simplification of the progressive alignment method of Feng & Doolittle, 1987, *J. Mol. Evol.* 35:351-360; the method is similar to that described by Higgins and Sharp, 1989, *CABIOS* 5:151-153. Useful PILEUP parameters including a default gap weight of 3.00, a default gap length weight of 0.10, and weighted end gaps.

[178] Another example of a useful algorithm is the BLAST algorithm, described in: Altschul *et al.*, 1990, *J. Mol. Biol.* 215:403-410; Altschul *et al.*, 1997, *Nucleic Acids Res.* 25:3389-3402; and Karin *et al.*, 1993, *Proc. Natl. Acad. Sci. U.S.A.* 90:5873-5787. A particularly useful BLAST program is the WU-BLAST-2 program which was obtained from Altschul *et al.*, 1996, *Methods in Enzymology* 266:460-480. WU-BLAST-2 uses several search parameters, most of which are set to the default values. The adjustable parameters are set with the following values: overlap span=1, overlap fraction=0.125, word threshold (T)=II. The HSP S and HSP S2 parameters are dynamic values and are established by the program itself depending upon the composition of the particular sequence and composition of the particular database against which the sequence of interest is being searched; however, the values may be adjusted to increase sensitivity.

[179] An additional useful algorithm is gapped BLAST as reported by Altschul *et al.*, 1993, *Nucl. Acids Res.* 25:3389-3402. Gapped BLAST uses BLOSUM-62 substitution scores; threshold T parameter set to 9; the two-hit method to trigger ungapped extensions, charges gap lengths of k a cost of 10+k; Xu set to 16, and Xg set to 40 for database search stage and to 67 for the output stage of the algorithms. Gapped alignments are triggered by a score corresponding to about 22 bits.

[180] Generally, the amino acid homology, similarity, or identity between individual variant CDRs or VH / VL sequences are at least 60% to the sequences depicted herein, and more typically with preferably increasing homologies or identities of at least 65% or 70%, more preferably at least 75% or 80%, even more preferably at least 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, and almost 100%. In a similar manner, "percent (%) nucleic acid sequence identity" with respect to the nucleic acid sequence of the binding proteins identified herein is defined as the percentage of nucleotide residues in a candidate sequence that are identical with the nucleotide residues in the coding sequence of the antigen-binding molecule. A specific method utilizes the BLASTN module of WU-BLAST-2 set to the default parameters, with overlap span and overlap fraction set to 1 and 0.125, respectively.

[181] Generally, the nucleic acid sequence homology, similarity, or identity between the nucleotide sequences encoding individual variant CDRs or VH / VL sequences and the nucleotide sequences depicted herein are at least 60%, and more typically with preferably increasing homologies or identities of at least 65%, 70%, 75%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%, and almost 100%. Thus, a "variant CDR" or a "variant VH / VL region"is one with the specified homology, similarity, or identity to the parent CDR / VH / VL of the invention, and shares biological function, including, but not limited to, at least 60%, 65%, 70%, 75%, 80%, 81%, 82%,

83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99% of the specificity and/or activity of the parent CDR or VH / VL.

[182] In one embodiment, the percentage of identity to human germline of the antigen-binding molecules according to the invention is ≥ 70% or ≥ 75%, more preferably ≥ 80% or ≥ 85%, even more preferably ≥ 90%, and most preferably ≥ 91%, ≥ 92%, ≥ 93%, ≥ 94%, ≥ 95% or even ≥ 96%. Identity to human antibody germline gene products is thought to be an important feature to reduce the risk of therapeutic proteins to elicit an immune response against the drug in the patient during treatment. Hwang & Foote ("Immunogenicity of engineered antibodies"; Methods 36 (2005) 3-10) demonstrate that the reduction of non-human portions of drug antigen-binding molecules leads to a decrease of risk to induce anti-drug antibodies in the patients during treatment. By comparing an exhaustive number of clinically evaluated antibody drugs and the respective immunogenicity data, the trend is shown that humanization of the V-regions of antibodies makes the protein less immunogenic (average 5.1 % of patients) than antibodies carrying unaltered non-human V regions (average 23.59 % of patients). A higher degree of identity to human sequences is hence desirable for V-region based protein therapeutics in the form of antigen-binding molecules. For this purpose of determining the germline identity, the V-regions of VL can be aligned with the amino acid sequences of human germline V segments and J segments (http://vbase.mrc-cpe.cam.ac.uk/) using Vector NTI software and the amino acid sequence calculated by dividing the identical amino acid residues by the total number of amino acid residues of the VL in percent. The same can be for the VH segments (http://vbase.mrc-cpe.cam.ac.uk/) with the exception that the VH CDR3 may be excluded due to its high diversity and a lack of existing human germline VH CDR3 alignment partners. Recombinant techniques can then be used to increase sequence identity to human antibody germline genes.

[183] In a further embodiment, the bispecific antigen-binding molecules of the present invention exhibit high monomer yields under standard research scale conditions, *e.g.,* in a standard two-step purification process. Preferably the monomer yield of the antigen-binding molecules according to the invention is ≥ 0.25 mg/L supernatant, more preferably ≥ 0.5 mg/L, even more preferably ≥ 1 mg/L, and most preferably ≥ 3 mg/L supernatant.

[184] Likewise, the yield of the dimeric antigen-binding molecule isoforms and hence the monomer percentage (*i.e.*, monomer : (monomer+dimer)) of the antigen-binding molecules can be determined. The productivity of monomeric and dimeric antigen-binding molecules and the calculated monomer percentage can *e.g.* be obtained in the SEC purification step of culture supernatant from standardized research-scale production in roller bottles. In one embodiment, the monomer percentage of the antigen-binding molecules is ≥ 80%, more preferably ≥ 85%, even more preferably ≥ 90%, and most preferably ≥ 95%.

[185] In one embodiment, the antigen-binding molecules have a preferred plasma stability (ratio of EC50 with plasma to EC50 w/o plasma) of ≤ 5 or ≤ 4, more preferably ≤ 3.5 or ≤ 3, even more preferably ≤ 2.5 or ≤ 2, and most preferably ≤ 1.5 or ≤ 1. The plasma stability of an antigen-binding molecule can be tested

by incubation of the construct in human plasma at 37°C for 24 hours followed by EC50 determination in a ^{51}chromium release cytotoxicity assay. The effector cells in the cytotoxicity assay can be stimulated enriched human CD8 positive T cells. Target cells can *e.g.* be CHO cells transfected with the human target cell surface antigen. The effector to target cell (E:T) ratio can be chosen as 10:1. The human plasma pool used for this purpose is derived from the blood of healthy donors collected by EDTA coated syringes. Cellular components are removed by centrifugation and the upper plasma phase is collected and subsequently pooled. As control, antigen-binding molecules are diluted immediately prior to the cytotoxicity assay in RPMI-1640 medium. The plasma stability is calculated as ratio of EC50 (after plasma incubation) to EC50 (control).

[186] It is furthermore preferred that the monomer to dimer conversion of antigen-binding molecules of the invention is low. The conversion can be measured under different conditions and analyzed by high performance size exclusion chromatography. For example, incubation of the monomeric isoforms of the antigen-binding molecules can be carried out for 7 days at 37°C and concentrations of *e.g.* 100 µg/ml or 250 µg/ml in an incubator. Under these conditions, it is preferred that the antigen-binding molecules of the invention show a dimer percentage that is ≤5%, more preferably ≤4%, even more preferably ≤3%, even more preferably ≤2.5%, even more preferably ≤2%, even more preferably ≤1.5%, and most preferably ≤1% or ≤0.5% or even 0%.

[187] It is also preferred that the bispecific antigen-binding molecules of the present invention present with very low dimer conversion after a number of freeze/thaw cycles. For example, the antigen-binding molecule monomer is adjusted to a concentration of 250 µg/ml *e.g.* in generic formulation buffer and subjected to three freeze/thaw cycles (freezing at -80°C for 30 min followed by thawing for 30 min at room temperature), followed by high performance SEC to determine the percentage of initially monomeric antigen-binding molecule, which had been converted into dimeric antigen-binding molecule. Preferably the dimer percentages of the bispecific antigen-binding molecules are ≤5%, more preferably ≤4%, even more preferably ≤3%, even more preferably ≤2.5%, even more preferably ≤2%, even more preferably ≤1.5%, and most preferably ≤1% or even ≤0.5%, for example after three freeze/thaw cycles.

[188] The bispecific antigen-binding molecules of the present invention preferably show a favorable thermostability with aggregation temperatures ≥45°C or ≥50°C, more preferably ≥52°C or ≥54°C, even more preferably ≥56°C or ≥57°C, and most preferably ≥58°C or ≥59°C. The thermostability parameter can be determined in terms of antibody aggregation temperature as follows: Antibody solution at a concentration 250 µg/ml is transferred into a single use cuvette and placed in a Dynamic Light Scattering (DLS) device. The sample is heated from 40°C to 70°C at a heating rate of 0.5°C/min with constant acquisition of the measured radius. Increase of radius indicating melting of the protein and aggregation is used to calculate the aggregation temperature of the antibody.

[189] Alternatively, temperature melting curves can be determined by Differential Scanning Calorimetry (DSC) to determine intrinsic biophysical protein stabilities of the antigen-binding molecules. These experiments are performed using a MicroCal LLC (Northampton, MA, U.S.A) VP-DSC device. The energy uptake of a sample containing an antigen-binding molecule is recorded from 20°C to 90°C compared to a sample containing only the formulation buffer. The antigen-binding molecules are adjusted to a final concentration of 250 µg/ml *e.g.* in SEC running buffer. For recording of the respective melting curve, the overall sample temperature is increased stepwise. At each temperature T energy uptake of the sample and the formulation buffer reference is recorded. The difference in energy uptake Cp (kcal/mole/°C) of the sample minus the reference is plotted against the respective temperature. The melting temperature is defined as the temperature at the first maximum of energy uptake.

[190] The target cell surface antigenxCD3 bispecific antigen-binding molecules of the invention are also envisaged to have a turbidity (as measured by OD340 after concentration of purified monomeric antigen-binding molecule to 2.5 mg/ml and over night incubation) of ≤ 0.2, preferably of ≤ 0.15, more preferably of ≤ 0.12, even more preferably of ≤ 0.1, and most preferably of ≤ 0.08.

[191] It is furthermore envisaged that the bispecific antigen-binding molecules of the present invention exhibit therapeutic efficacy or anti-tumor activity. This can *e.g.* be assessed in a study as disclosed in the following example of an advanced stage human tumor xenograft model:

[192] The skilled person knows how to modify or adapt certain parameters of this study, such as the number of injected tumor cells, the site of injection, the number of transplanted human T cells, the amount of bispecific antigen-binding molecules to be administered, and the timelines, while still arriving at a meaningful and reproducible result. Preferably, the tumor growth inhibition T/C [%] is ≤ 70 or ≤ 60, more preferably ≤ 50 or ≤ 40, even more preferably ≤ 30 or ≤ 20 and most preferably ≤ 10 or ≤ 5 or even ≤ 2.5.

[193] In a preferred embodiment of the antigen-binding molecule of the invention the antigen-binding molecule is a single chain antigen-binding molecule.

[194] Also in a preferred embodiment of the antigen-binding molecule of the invention said third domain comprises in an amino to carboxyl order:

hinge-CH2-CH3-linker-hinge-CH2-CH3.

[195] Also in one embodiment of the invention the CH2 domain of one or preferably each (both) polypeptide monomers of the third domain comprises an intra domain cysteine disulfide bridge. As known in the art the term "cysteine disulfide bridge" refers to a functional group with the general structure *R–S–S–R*. The linkage is also called an SS-bond or a disulfide bridge and is derived by the coupling of two thiol groups of cysteine residues. It is particularly preferred for the antigen-binding molecule of the invention that the cysteines forming the cysteine disulfide bridge in the mature antigen-binding molecule are

introduced into the amino acid sequence of the CH2 domain corresponding to 309 and 321 (Kabat numbering).

[196] In one embodiment of the invention a glycosylation site in Kabat position 314 of the CH2 domain is removed. It is preferred that this removal of the glycosylation site is achieved by a N314X substitution, wherein X is any amino acid excluding Q. Said substitution is preferably a N314G substitution. In a more preferred embodiment, said CH2 domain additionally comprises the following substitutions (position according to Kabat) V321C and R309C (these substitutions introduce the intra domain cysteine disulfide bridge at Kabat positions 309 and 321).

[197] It is assumed that the preferred features of the antigen-binding molecule of the invention compared e.g. to the bispecific heteroFc antigen-binding molecule known in the art (figure 1b) may be inter alia related to the introduction of the above described modifications in the CH2 domain. Thus, it is preferred for the construct of the invention that the CH2 domains in the third domain of the antigen-binding molecule of the invention comprise the intra domain cysteine disulfide bridge at Kabat positions 309 and 321 and/or the glycosylation site at Kabat position 314 is removed by a N314X substitution as above, preferably by a N314G substitution.

[198] In a further preferred embodiment of the invention the CH2 domains in the third domain of the antigen-binding molecule of the invention comprise the intra domain cysteine disulfide bridge at Kabat positions 309 and 321 and the glycosylation site at Kabat position 314 is removed by a N314G substitution.

[199] In one embodiment the invention provides an antigen-binding molecule, wherein:

(182) the first domain comprises two antibody variable domains and the second domain comprises two antibody variable domains;

(ii) the first domain comprises one antibody variable domain and the second domain comprises two antibody variable domains;

(iii) the first domain comprises two antibody variable domains and the second domain comprises one antibody variable domain; or

(iv) the first domain comprises one antibody variable domain and the second domain comprises one antibody variable domain.

[200] Accordingly, the first and the second domain may be binding domains comprising each two antibody variable domains such as a VH and a VL domain. Examples for such binding domains comprising two antibody variable domains where described herein above and comprise e.g. Fv fragments, scFv fragments or Fab fragments described herein above. Alternatively either one or both of those binding domains may comprise only a single variable domain. Examples for such single domain binding domains where described herein above and comprise e.g. nanobodies or single variable domain antibodies comprising merely one variable domain, which might be VHH, VH or VL, that specifically bind an antigen or epitope independently of other V regions or domains.

[201] In a preferred embodiment of the antigen-binding molecule of the invention first and second domain are fused to the third domain via a peptide linker. Preferred peptide linker have been described herein above and are characterized by the amino acid sequence Gly-Gly-Gly-Gly-Ser, i.e. Gly_4Ser (SEQ ID NO: 187), or polymers thereof, i.e. $(Gly_4Ser)x$, where x is an integer of 1 or greater (e.g. 2 or 3). A particularly preferred linker for the fusion of the first and second domain to the third domain is depicted in SEQ ID Nos: 1.

[202] In a preferred embodiment the antigen-binding molecule of the invention is characterized to comprise in an amino to carboxyl order:

(a) the first domain;

(b) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID Nos: 187-189;

I the second domain;

(d) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NO: 187, 188, 189, 195, 196, 197 and 198;

I the first polypeptide monomer of the third domain;

(f) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID Nos: 191, 192, 193 and 194; and

(g) the second polypeptide monomer of the third domain.

[203] In one aspect of the invention the target cell surface antigen bound by the first domain is a tumor antigen, an antigen specific for an immunological disorder or a viral antigen. The term "tumor antigen" as used herein may be understood as those antigens that are presented on tumor cells. These antigens can be presented on the cell surface with an extracellular part, which is often combined with a transmembrane and cytoplasmic part of the molecule. These antigens can sometimes be presented only by tumor cells and never by the normal ones. Tumor antigens can be exclusively expressed on tumor cells or might represent a tumor specific mutation compared to normal cells. In this case, they are called tumor-specific antigens. More common are antigens that are presented by tumor cells and normal cells, and they are called tumor-associated antigens. These tumor-associated antigens can be overexpressed compared to normal cells or are accessible for antibody binding in tumor cells due to the less compact structure of the tumor tissue compared to normal tissue. Non-limiting examples of tumor antigens as used herein are CDH19, MSLN, DLL3, FLT3, EGFRvIII, CD33, CD19, MUC17, CLDN18.2, CDH3, CD70, BCMA and PSMA.

[204] Further target cell surface antigens specific for an immunological disorder in the context of the present invention comprise, for example, TL1A and TNF-alpha. Said targets are preferably addressed by a bispecific antigen-binding molecule of the present invention, which is preferably a full length antibody. In a very preferred embodiment, an antibody of the present invention is a hetero IgG antibody.

[205] In a preferred embodiment of the antigen-binding molecule of the invention the tumor antigen, preferably tumor antigen, is selected from the group consisting of CDH19, MSLN, DLL3, FLT3, EGFRvIII, CD33, CD19, , MUC17, CLDN18.2, CDH3, CD70, BCMA and PSMA.

[206] In one aspect of the invention the antigen-binding molecule comprises in an amino to carboxyl order:

(a) the first domain having an amino acid sequence selected from the group consisting of SEQ ID Nos: 7, 8, 17, 27, 28, 37, 38, 39, 40, 41, 48, 49, 50, 51,52, 59, 60, 61, 62, 63, 64, 71, 72, 73, 74, 75. 76, 77, 78, 79, 80, 81, 89, 90, 91, 92, 93, 100, 101, 102, 103, 104, 113, 114, 121, 122,123, 124, 125, 131, 132, 133, 134, 135, 136, 143, 144, 145, 146, 147, 148, 149, 150, 151, 158, 159, 160, 161, 162, 163, 164, 165, 166, 173, 174, 175, 176, 177, 178, 179, 180, 181, 223, 235 and 246,

(b) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID Nos: 187-189;

I the second domain having an amino acid sequence selected from the group consisting of SEQ ID Nos: SEQ ID Nos: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187 of WO 2008/119567 or of SEQ ID NO: 202;

(d) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID Nos: 187, 188, 189, 195, 196, 197 and 198;

I the first polypeptide monomer of the third domain having a polypeptide sequence selected from the group consisting of SEQ ID Nos: 17-24 of WO2017/134140;

(f) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID Nos: 191, 192, 193 and 194; and

(g) the second polypeptide monomer of the third domain having a polypeptide sequence selected from the group consisting of SEQ ID Nos: 17-24 of WO2017/134140 .

[207] In one aspect, the bispecific antigen-binding molecule of the invention is characterized by having an amino acid sequence selected from the group consisting of and being directed to the respective target cell surface antigen:

(a) SEQ ID Nos: 39 to 41; CD33
(b) SEQ ID Nos: each of 48 to 52; EGFRvIII
(c) SEQ ID Nos: each of 59 to 64; MSLN
(d) SEQ ID Nos: each of 71 to 82 CDH19
(e) SEQ ID Nos: each of 100 to 104 DLL3
(f) SEQ ID Nos: 7, 8, 17, 113 and 114CD19
(g) SEQ ID Nos: each of 89 to 93 FLT3
(h) SEQ ID Nos: each of 121 to 125 CDH3
(i) SEQ ID Nos: each of 132 to 136 BCMA
(j) SEQ ID Nos: each of 143 to 151, 158 to 166 and 173 to 181 PSMA

(k) SEQ ID NO 213	MUC17
(l) SEQ ID NOs: each of 225 and 237	CLDN18.2
(m) SEQ ID No: 248	CD70 and
(n) SEQ ID NO 258 and 261	CLDN 6

[208] The invention further provides a polynucleotide / nucleic acid molecule encoding an antigen-binding molecule of the invention. A polynucleotide is a biopolymer composed of 13 or more nucleotide monomers covalently bonded in a chain. DNA (such as cDNA) and RNA (such as mRNA) are examples of polynucleotides with distinct biological function. Nucleotides are organic molecules that serve as the monomers or subunits of nucleic acid molecules like DNA or RNA. The nucleic acid molecule or polynucleotide can be double stranded and single stranded, linear and circular. It is preferably comprised in a vector which is preferably comprised in a host cell. Said host cell is, *e.g.* after transformation or transfection with the vector or the polynucleotide of the invention, capable of expressing the antigen-binding molecule. For that purpose the polynucleotide or nucleic acid molecule is operatively linked with control sequences.

[209] The genetic code is the set of rules by which information encoded within genetic material (nucleic acids) is translated into proteins. Biological decoding in living cells is accomplished by the ribosome which links amino acids in an order specified by mRNA, using tRNA molecules to carry amino acids and to read the mRNA three nucleotides at a time. The code defines how sequences of these nucleotide triplets, called codons, specify which amino acid will be added next during protein synthesis. With some exceptions, a three-nucleotide codon in a nucleic acid sequence specifies a single amino acid. Because the vast majority of genes are encoded with exactly the same code, this particular code is often referred to as the canonical or standard genetic code. While the genetic code determines the protein sequence for a given coding region, other genomic regions can influence when and where these proteins are produced.

[210] Furthermore, the invention provides a vector comprising a polynucleotide / nucleic acid molecule of the invention. A vector is a nucleic acid molecule used as a vehicle to transfer (foreign) genetic material into a cell. The term "vector" encompasses – but is not restricted to – plasmids, viruses, cosmids and artificial chromosomes. In general, engineered vectors comprise an origin of replication, a multicloning site and a selectable marker. The vector itself is generally a nucleotide sequence, commonly a DNA sequence that comprises an insert (transgene) and a larger sequence that serves as the "backbone" of the vector. Modern vectors may encompass additional features besides the transgene insert and a backbone: promoter, genetic marker, antibiotic resistance, reporter gene, targeting sequence, protein purification tag. Vectors called expression vectors (expression constructs) specifically are for the expression of the transgene in the target cell, and generally have control sequences.

[211] The term "control sequences" refers to DNA sequences necessary for the expression of an operably linked coding sequence in a particular host organism. The control sequences that are suitable for prokaryotes, for example, include a promoter, optionally an operator sequence, and a ribosome binding side. Eukaryotic cells are known to utilize promoters, polyadenylation signals, and enhancers.

[212] A nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding sequence if it affects the transcription of the sequence; or a ribosome binding side is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[213] "Transfection" is the process of deliberately introducing nucleic acid molecules or polynucleotides (including vectors) into target cells. The term is mostly used for non-viral methods in eukaryotic cells. Transduction is often used to describe virus-mediated transfer of nucleic acid molecules or polynucleotides. Transfection of animal cells typically involves opening transient pores or "holes" in the cell membrane, to allow the uptake of material. Transfection can be carried out using calcium phosphate, by electroporation, by cell squeezing or by mixing a cationic lipid with the material to produce liposomes, which fuse with the cell membrane and deposit their cargo inside.

[214] The term "transformation" is used to describe non-viral transfer of nucleic acid molecules or polynucleotides (including vectors) into bacteria, and also into non-animal eukaryotic cells, including plant cells. Transformation is hence the genetic alteration of a bacterial or non-animal eukaryotic cell resulting from the direct uptake through the cell membrane(s) from its surroundings and subsequent incorporation of exogenous genetic material (nucleic acid molecules). Transformation can be effected by artificial means. For transformation to happen, cells or bacteria must be in a state of competence, which might occur as a time-limited response to environmental conditions such as starvation and cell density.

[215] Moreover, the invention provides a host cell transformed or transfected with the polynucleotide / nucleic acid molecule or with the vector of the invention. As used herein, the terms "host cell" or "recipient cell" are intended to include any individual cell or cell culture that can be or has/have been recipients of vectors, exogenous nucleic acid molecules, and polynucleotides encoding the antigen-binding molecule of the present invention; and/or recipients of the antigen-binding molecule itself. The introduction of the respective material into the cell is carried out by way of transformation, transfection and the like. The term "host cell" is also intended to include progeny or potential progeny of a single cell. Because certain modifications may occur in succeeding generations due to either natural, accidental, or deliberate mutation

or due to environmental influences, such progeny may not, in fact, be completely identical (in morphology or in genomic or total DNA complement) to the parent cell, but is still included within the scope of the term as used herein. Suitable host cells include prokaryotic or eukaryotic cells, and also include but are not limited to bacteria, yeast cells, fungi cells, plant cells, and animal cells such as insect cells and mammalian cells, e.g., murine, rat, macaque or human.

[216] The antigen-binding molecule of the invention can be produced in bacteria. After expression, the antigen-binding molecule of the invention is isolated from the *E. coli* cell paste in a soluble fraction and can be purified through, e.g., affinity chromatography and/or size exclusion. Final purification can be carried out similar to the process for purifying antibody expressed *e.g.*, in CHO cells.

[217] In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for the antigen-binding molecule of the invention. *Saccharomyces cerevisiae*, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as *Schizosaccharomyces pombe*, Kluyveromyces hosts such as *K. lactis, K. fragilis* (ATCC 12424), *K. bulgaricus* (ATCC 16045), *K. wickeramii* (ATCC 24178), *K. waltii* (ATCC 56500), *K. drosophilarum* (ATCC 36906), *K. thermotolerans*, and *K. marxianus*; yarrowia (EP 402 226); *Pichia pastoris* (EP 183 070); Candida; *Trichoderma reesia* (EP 244 234); *Neurospora crassa*; Schwanniomyces such as *Schwanniomyces occidentalis*; and filamentous fungi such as Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts such as *A. nidulans* and *A. niger*.

[218] Suitable host cells for the expression of glycosylated antigen-binding molecule of the invention are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as *Spodoptera frugiperda* (caterpillar), *Aedes aegypti* (mosquito), *Aedes albopictus* (mosquito), *Drosophila melanogaster* (fruit fly), and *Bombyx mori* have been identified. A variety of viral strains for transfection are publicly available, *e.g.*, the L-1 variant of *Autographa californica* NPV and the Bm-5 strain of *Bombyx mori* NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of *Spodoptera frugiperda* cells.

[219] Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, Arabidopsis and tobacco can also be used as hosts. Cloning and expression vectors useful in the production of proteins in plant cell culture are known to those of skill in the art. See *e.g.* Hiatt *et al.*, Nature (1989) 342: 76-78, Owen *et al.* (1992) Bio/Technology 10: 790-794, Artsaenko *et al.* (1995) The Plant J 8: 745-750, and Fecker *et al.* (1996) Plant Mol Biol 32: 979-986.

[220] However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are

monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651); human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al. , J. Gen Virol. 36 : 59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub *et al.*, Proc. Natl. Acad. Sci. USA 77: 4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23: 243-251 (1980)); monkey kidney cells (CVI ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL1587); human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2,1413 8065); mouse mammary tumor (MMT 060562, ATCC CCL5 1); TRI cells (Mather *et al.*, Annals N. Y Acad. Sci. (1982) 383: 44-68); MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[221] In a further embodiment the invention provides a process for the production of an antigen-binding molecule of the invention, said process comprising culturing a host cell of the invention under conditions allowing the expression of the antigen-binding molecule of the invention and recovering the produced antigen-binding molecule from the culture.

[222] As used herein, the term "culturing" refers to the *in vitro* maintenance, differentiation, growth, proliferation and/or propagation of cells under suitable conditions in a medium. The term "expression" includes any step involved in the production of an antigen-binding molecule of the invention including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

[223] When using recombinant techniques, the antigen-binding molecule can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the antigen-binding molecule is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter *et al.*, Bio/Technology 10: 163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of *E. coli*. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[224] The antigen-binding molecule of the invention prepared from the host cells can be recovered or purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on

heparin SEPHAROSE™, chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromato-focusing SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered. Where the antigen-binding molecule of the invention comprises a CH3 domain, the Bakerbond ABX resin (J.T. Baker, Phillipsburg, NJ) is useful for purification.

[225] Affinity chromatography is a preferred purification technique. The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly (styrenedivinyl) benzene allow for faster flow rates and shorter processing times than can be achieved with agarose.

[226] Moreover, the invention provides a pharmaceutical composition comprising an antigen-binding molecule of the invention or an antigen-binding molecule produced according to the process of the invention. It is preferred for the pharmaceutical composition of the invention that the homogeneity of the antigen-binding molecule is ≥ 80%, more preferably ≥ 81%,≥ 82%,≥ 83%,≥ 84%, or ≥ 85%, further preferably ≥ 86%,≥ 87%,≥ 88%,≥ 89%, or ≥ 90%, still further preferably, ≥ 91%,≥ 92%,≥ 93%,≥ 94%, or ≥ 95% and most preferably ≥ 96%,≥ 97%,≥ 98% or ≥ 99%.

[227] As used herein, the term "pharmaceutical composition" relates to a composition which is suitable for administration to a patient, preferably a human patient. The particularly preferred pharmaceutical composition of this invention comprises one or a plurality of the antigen-binding molecule(s) of the invention, preferably in a therapeutically effective amount. Preferably, the pharmaceutical composition further comprises suitable formulations of one or more (pharmaceutically effective) carriers, stabilizers, excipients, diluents, solubilizers, surfactants, emulsifiers, preservatives and/or adjuvants. Acceptable constituents of the composition are preferably nontoxic to recipients at the dosages and concentrations employed. Pharmaceutical compositions of the invention include, but are not limited to, liquid, frozen, and lyophilized compositions.

[228] The inventive compositions may comprise a pharmaceutically acceptable carrier. In general, as used herein, "pharmaceutically acceptable carrier" means any and all aqueous and non-aqueous solutions, sterile solutions, solvents, buffers, e.g. phosphate buffered saline (PBS) solutions, water, suspensions, emulsions, such as oil/water emulsions, various types of wetting agents, liposomes, dispersion media and coatings, which are compatible with pharmaceutical administration, in particular with parenteral administration. The use of such media and agents in pharmaceutical compositions is well known in the art, and the compositions comprising such carriers can be formulated by well-known conventional methods.

[229] Certain embodiments provide pharmaceutical compositions comprising the antigen-binding molecule of the invention and further one or more excipients such as those illustratively described in this section and elsewhere herein. Excipients can be used in the invention in this regard for a wide variety of purposes, such as adjusting physical, chemical, or biological properties of formulations, such as adjustment

of viscosity, and or processes of the invention to improve effectiveness and or to stabilize such formulations and processes against degradation and spoilage due to, for instance, stresses that occur during manufacturing, shipping, storage, pre-use preparation, administration, and thereafter.

[230] In certain embodiments, the pharmaceutical composition may contain formulation materials for the purpose of modifying, maintaining or preserving, e.g., the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition (see, REMINGTON'S PHARMACEUTICAL SCIENCES, 18" Edition, (A.R. Genrmo, ed.), 1990, Mack Publishing Company). In such embodiments, suitable formulation materials may include, but are not limited to:

- amino acids such as glycine, alanine, glutamine, asparagine, threonine, proline, 2-phenylalanine, including charged amino acids, preferably lysine, lysine acetate, arginine, glutamate and/or histidine
- antimicrobials such as antibacterial and antifungal agents
- antioxidants such as ascorbic acid, methionine, sodium sulfite or sodium hydrogen-sulfite;
- buffers, buffer systems and buffering agents which are used to maintain the composition at physiological pH or at a slightly lower pH; examples of buffers are borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids, succinate, phosphate, and histidine; for example Tris buffer of about pH 7.0-8.5;
- non-aqueous solvents such as propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate;
- aqueous carriers including water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media;
- biodegradable polymers such as polyesters;
- bulking agents such as mannitol or glycine;
- chelating agents such as ethylenediamine tetraacetic acid (EDTA);
- isotonic and absorption delaying agents;
- complexing agents such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin)
- fillers;
- monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrins); carbohydrates may be non-reducing sugars, preferably trehalose, sucrose, octasulfate, sorbitol or xylitol;
- (low molecular weight) proteins, polypeptides or proteinaceous carriers such as human or bovine serum albumin, gelatin or immunoglobulins, preferably of human origin;
- coloring and flavouring agents;
- sulfur containing reducing agents, such as glutathione, thioctic acid, sodium thioglycolate, thioglycerol, [alpha]-monothioglycerol, and sodium thio sulfate

- diluting agents;
- emulsifying agents;
- hydrophilic polymers such as polyvinylpyrrolidone)
- salt-forming counter-ions such as sodium;
- preservatives such as antimicrobials, anti-oxidants, chelating agents, inert gases and the like; examples are: benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide);
- metal complexes such as Zn-protein complexes;
- solvents and co-solvents (such as glycin, propylene glycol or polyethylene glycol);
- sugars and sugar alcohols, such as trehalose, sucrose, octasulfate, mannitol, sorbitol or xylitol stachyose, mannose, sorbose, xylose, ribose, myoinisitose, galactose, lactitol, ribitol, myoinisitol, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; and polyhydric sugar alcohols;
- suspending agents;
- surfactants or wetting agents such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate, triton, tromethamine, lecithin, cholesterol, tyloxapal; surfactants may be detergents, preferably with a molecular weight of >1.2 KD and/or a polyether, preferably with a molecular weight of >3 KD; non-limiting examples for preferred detergents are Tween 20, Tween 40, Tween 60, Tween 80 and Tween 85; non-limiting examples for preferred polyethers are PEG 3000, PEG 3350, PEG 4000 and PEG 5000;
- stability enhancing agents such as sucrose or sorbitol;
- tonicity enhancing agents such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol;
- parenteral delivery vehicles including sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils;
- intravenous delivery vehicles including fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose).

[231] It is evident to those skilled in the art that the different constituents of the pharmaceutical composition (e.g., those listed above) can have different effects, for example, and amino acid can act as a buffer, a stabilizer and/or an antioxidant; mannitol can act as a bulking agent and/or a tonicity enhancing agent; sodium chloride can act as delivery vehicle and/or tonicity enhancing agent; etc.

[232] It is envisaged that the composition of the invention might comprise, in addition to the polypeptide of the invention defined herein, further biologically active agents, depending on the intended use of the composition. Such agents might be drugs acting on the gastro-intestinal system, drugs acting as cytostatica, drugs preventing hyperurikemia, drugs inhibiting immunoreactions (e.g. corticosteroids), drugs modulating the inflammatory response, drugs acting on the circulatory system and/or agents such as

cytokines known in the art. It is also envisaged that the antigen-binding molecule of the present invention is applied in a co-therapy, i.e., in combination with another anti-cancer medicament.

[233] In certain embodiments, the optimal pharmaceutical composition will be determined by one skilled in the art depending upon, for example, the intended route of administration, delivery format and desired dosage. See, for example, REMINGTON'S PHARMACEUTICAL SCIENCES, supra. In certain embodiments, such compositions may influence the physical state, stability, rate of in vivo release and rate of in vivo clearance of the antigen-binding molecule of the invention. In certain embodiments, the primary vehicle or carrier in a pharmaceutical composition may be either aqueous or non-aqueous in nature. For example, a suitable vehicle or carrier may be water for injection, physiological saline solution or artificial cerebrospinal fluid, possibly supplemented with other materials common in compositions for parenteral administration. Neutral buffered saline or saline mixed with serum albumin are further exemplary vehicles. In certain embodiments, the antigen-binding molecule of the invention compositions may be prepared for storage by mixing the selected composition having the desired degree of purity with optional formulation agents (REMINGTON'S PHARMACEUTICAL SCIENCES, supra) in the form of a lyophilized cake or an aqueous solution. Further, in certain embodiments, the antigen-binding molecule of the invention may be formulated as a lyophilizate using appropriate excipients such as sucrose.

[234] When parenteral administration is contemplated, the therapeutic compositions for use in this invention may be provided in the form of a pyrogen-free, parenterally acceptable aqueous solution comprising the desired antigen-binding molecule of the invention in a pharmaceutically acceptable vehicle. A particularly suitable vehicle for parenteral injection is sterile distilled water in which the antigen-binding molecule of the invention is formulated as a sterile, isotonic solution, properly preserved. In certain embodiments, the preparation can involve the formulation of the desired molecule with an agent, such as injectable microspheres, bio-erodible particles, polymeric compounds (such as polylactic acid or polyglycolic acid), beads or liposomes, that may provide controlled or sustained release of the product which can be delivered via depot injection. In certain embodiments, hyaluronic acid may also be used, having the effect of promoting sustained duration in the circulation. In certain embodiments, implantable drug delivery devices may be used to introduce the desired antigen-binding molecule.

[235] Additional pharmaceutical compositions will be evident to those skilled in the art, including formulations involving the antigen-binding molecule of the invention in sustained- or controlled-delivery / release formulations. Techniques for formulating a variety of other sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. See, for example, International Patent Application No. PCT/US93/00829, which describes controlled release of porous polymeric microparticles for delivery of pharmaceutical compositions. Sustained-release preparations may include semipermeable polymer matrices in the form of shaped articles, e.g., films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides (as disclosed in U.S. Pat. No. 3,773,919 and European Patent

Application Publication No. EP 058481), copolymers of L-glutamic acid and gamma ethyl-L-glutamate (Sidman et al., 1983, Biopolymers 2:547-556), poly (2-hydroxyethyl-methacrylate) (Langer et al., 1981, J. Biomed. Mater. Res. 15:167-277 and Langer, 1982, Chem. Tech. 12:98-105), ethylene vinyl acetate (Langer et al., 1981, supra) or poly-D(-)-3-hydroxybutyric acid (European Patent Application Publication No. EP 133,988). Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art. See, e.g., Eppstein et al., 1985, Proc. Natl. Acad. Sci. U.S.A. 82 :3688-3692 ; European Patent Application Publication Nos. EP 036,676; EP 088,046 and EP 143,949.

[236] The antigen-binding molecule may also be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly (methylmethacylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nanoparticles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

[237] Pharmaceutical compositions used for in vivo administration are typically provided as sterile preparations. Sterilization can be accomplished by filtration through sterile filtration membranes. When the composition is lyophilized, sterilization using this method may be conducted either prior to or following lyophilisation and reconstitution. Compositions for parenteral administration can be stored in lyophilized form or in a solution. Parenteral compositions generally are placed into a container having a sterile access port, for example, an intravenous solution bag or vial having a stopper pierceable by a hypodermic injection needle.

[238] Another aspect of the invention includes self-buffering antigen-binding molecule of the invention formulations, which can be used as pharmaceutical compositions, as described in international patent application WO 06138181A2 (PCT/US2006/022599). A variety of expositions are available on protein stabilization and formulation materials and methods useful in this regard, such as Arakawa et al., "Solvent interactions in pharmaceutical formulations," Pharm Res. 8(3): 285-91 (1991); Kendrick et al., "Physical stabilization of proteins in aqueous solution" in: RATIONAL DESIGN OF STABLE PROTEIN FORMULATIONS: THEORY AND PRACTICE, Carpenter and Manning, eds. Pharmaceutical Biotechnology. 13: 61-84 (2002), and Randolph et al., "Surfactant-protein interactions", Pharm Biotechnol. 13: 159-75 (2002), see particularly the parts pertinent to excipients and processes of the same for self-buffering protein formulations in accordance with the current invention, especially as to protein pharmaceutical products and processes for veterinary and/or human medical uses.

[239] Salts may be used in accordance with certain embodiments of the invention to, for example, adjust the ionic strength and/or the isotonicity of a formulation and/or to improve the solubility and/or physical stability of a protein or other ingredient of a composition in accordance with the invention. As is well known, ions can stabilize the native state of proteins by binding to charged residues on the protein's surface

and by shielding charged and polar groups in the protein and reducing the strength of their electrostatic interactions, attractive, and repulsive interactions. Ions also can stabilize the denatured state of a protein by binding to, in particular, the denatured peptide linkages (--CONH) of the protein. Furthermore, ionic interaction with charged and polar groups in a protein also can reduce intermolecular electrostatic interactions and, thereby, prevent or reduce protein aggregation and insolubility.

[240] Ionic species differ significantly in their effects on proteins. A number of categorical rankings of ions and their effects on proteins have been developed that can be used in formulating pharmaceutical compositions in accordance with the invention. One example is the Hofmeister series, which ranks ionic and polar non-ionic solutes by their effect on the conformational stability of proteins in solution. Stabilizing solutes are referred to as "kosmotropic". Destabilizing solutes are referred to as "chaotropic". Kosmotropes commonly are used at high concentrations (e.g., >1 molar ammonium sulfate) to precipitate proteins from solution ("salting-out"). Chaotropes commonly are used to denture and/or to solubilize proteins ("salting-in"). The relative effectiveness of ions to "salt-in" and "salt-out" defines their position in the Hofmeister series.

[241] Free amino acids can be used in the antigen-binding molecule of the invention formulations in accordance with various embodiments of the invention as bulking agents, stabilizers, and antioxidants, as well as other standard uses. Lysine, proline, serine, and alanine can be used for stabilizing proteins in a formulation. Glycine is useful in lyophilisation to ensure correct cake structure and properties. Arginine may be useful to inhibit protein aggregation, in both liquid and lyophilized formulations. Methionine is useful as an antioxidant.

[242] Polyols include sugars, e.g., mannitol, sucrose, and sorbitol and polyhydric alcohols such as, for instance, glycerol and propylene glycol, and, for purposes of discussion herein, polyethylene glycol (PEG) and related substances. Polyols are kosmotropic. They are useful stabilizing agents in both liquid and lyophilized formulations to protect proteins from physical and chemical degradation processes. Polyols also are useful for adjusting the tonicity of formulations. Among polyols useful in select embodiments of the invention is mannitol, commonly used to ensure structural stability of the cake in lyophilized formulations. It ensures structural stability to the cake. It is generally used with a lyoprotectant, e.g., sucrose. Sorbitol and sucrose are among preferred agents for adjusting tonicity and as stabilizers to protect against freeze-thaw stresses during transport or the preparation of bulks during the manufacturing process. Reducing sugars (which contain free aldehyde or ketone groups), such as glucose and lactose, can glycate surface lysine and arginine residues. Therefore, they generally are not among preferred polyols for use in accordance with the invention. In addition, sugars that form such reactive species, such as sucrose, which is hydrolyzed to fructose and glucose under acidic conditions, and consequently engenders glycation, also is not among preferred polyols of the invention in this regard. PEG is useful to stabilize proteins and as a cryoprotectant and can be used in the invention in this regard.

[243] Embodiments of the antigen-binding molecule of the invention formulations further comprise surfactants. Protein molecules may be susceptible to adsorption on surfaces and to denaturation and consequent aggregation at air-liquid, solid-liquid, and liquid-liquid interfaces. These effects generally scale inversely with protein concentration. These deleterious interactions generally scale inversely with protein concentration and typically are exacerbated by physical agitation, such as that generated during the shipping and handling of a product. Surfactants routinely are used to prevent, minimize, or reduce surface adsorption. Useful surfactants in the invention in this regard include polysorbate 20, polysorbate 80, other fatty acid esters of sorbitan polyethoxylates, and poloxamer 188. Surfactants also are commonly used to control protein conformational stability. The use of surfactants in this regard is protein-specific since, any given surfactant typically will stabilize some proteins and destabilize others.

[244] Polysorbates are susceptible to oxidative degradation and often, as supplied, contain sufficient quantities of peroxides to cause oxidation of protein residue side-chains, especially methionine. Consequently, polysorbates should be used carefully, and when used, should be employed at their lowest effective concentration. In this regard, polysorbates exemplify the general rule that excipients should be used in their lowest effective concentrations.

[245] Embodiments of the antigen-binding molecule of the invention formulations further comprise one or more antioxidants. To some extent deleterious oxidation of proteins can be prevented in pharmaceutical formulations by maintaining proper levels of ambient oxygen and temperature and by avoiding exposure to light. Antioxidant excipients can be used as well to prevent oxidative degradation of proteins. Among useful antioxidants in this regard are reducing agents, oxygen/free-radical scavengers, and chelating agents. Antioxidants for use in therapeutic protein formulations in accordance with the invention preferably are water-soluble and maintain their activity throughout the shelf life of a product. EDTA is a preferred antioxidant in accordance with the invention in this regard. Antioxidants can damage proteins. For instance, reducing agents, such as glutathione in particular, can disrupt intramolecular disulfide linkages. Thus, antioxidants for use in the invention are selected to, among other things, eliminate or sufficiently reduce the possibility of themselves damaging proteins in the formulation.

[246] Formulations in accordance with the invention may include metal ions that are protein co-factors and that are necessary to form protein coordination complexes, such as zinc necessary to form certain insulin suspensions. Metal ions also can inhibit some processes that degrade proteins. However, metal ions also catalyze physical and chemical processes that degrade proteins. Magnesium ions (10-120 mM) can be used to inhibit isomerization of aspartic acid to isoaspartic acid. Ca^{+2} ions (up to 100 mM) can increase the stability of human deoxyribonuclease. Mg^{+2}, Mn^{+2}, and Zn^{+2}, however, can destabilize rhDNase. Similarly, Ca^{+2} and Sr^{+2} can stabilize Factor VIII, it can be destabilized by Mg^{+2}, Mn^{+2} and Zn^{+2}, Cu^{+2} and Fe^{+2}, and its aggregation can be increased by Al^{+3} ions.

[247] Embodiments of the antigen-binding molecule of the invention formulations further comprise one or more preservatives. Preservatives are necessary when developing multi-dose parenteral formulations that involve more than one extraction from the same container. Their primary function is to inhibit microbial growth and ensure product sterility throughout the shelf-life or term of use of the drug product. Commonly used preservatives include benzyl alcohol, phenol and m-cresol. Although preservatives have a long history of use with small-molecule parenterals, the development of protein formulations that includes preservatives can be challenging. Preservatives almost always have a destabilizing effect (aggregation) on proteins, and this has become a major factor in limiting their use in multi-dose protein formulations. To date, most protein drugs have been formulated for single-use only. However, when multi-dose formulations are possible, they have the added advantage of enabling patient convenience, and increased marketability. A good example is that of human growth hormone (hGH) where the development of preserved formulations has led to commercialization of more convenient, multi-use injection pen presentations. At least four such pen devices containing preserved formulations of hGH are currently available on the market. Norditropin (liquid, Novo Nordisk), Nutropin AQ (liquid, Genentech) & Genotropin (lyophilized–dual chamber cartridge, Pharmacia & Upjohn) contain phenol while Somatrope (Eli Lilly) is formulated with m-cresol. Several aspects need to be considered during the formulation and development of preserved dosage forms. The effective preservative concentration in the drug product must be optimized. This requires testing a given preservative in the dosage form with concentration ranges that confer anti-microbial effectiveness without compromising protein stability.

[248] As might be expected, development of liquid formulations containing preservatives are more challenging than lyophilized formulations. Freeze-dried products can be lyophilized without the preservative and reconstituted with a preservative containing diluent at the time of use. This shortens the time for which a preservative is in contact with the protein, significantly minimizing the associated stability risks. With liquid formulations, preservative effectiveness and stability should be maintained over the entire product shelf-life (about 18 to 24 months). An important point to note is that preservative effectiveness should be demonstrated in the final formulation containing the active drug and all excipient components.

[249] The antigen-binding molecules disclosed herein may also be formulated as liposomes. A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Liposomes containing the antigen-binding molecule are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al. , Proc. Natl Acad. Sci. USA, 77: 4030 (1980); US Pat. Nos. 4,485,045 and 4,544,545; and W0 97/38731. Liposomes with enhanced circulation time are disclosed in US Patent No. 5,013, 556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-

derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antigen-binding molecule of the present invention can be conjugated to the liposomes as described in Martin et al. J. Biol. Chem. 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon et al. J. National Cancer Inst. 81 (19) 1484 (1989).

[250] Once the pharmaceutical composition has been formulated, it may be stored in sterile vials as a solution, suspension, gel, emulsion, solid, crystal, or as a dehydrated or lyophilized powder. Such formulations may be stored either in a ready-to-use form or in a form (e.g., lyophilized) that is reconstituted prior to administration.

[251] The biological activity of the pharmaceutical composition defined herein can be determined for instance by cytotoxicity assays, as described in the following examples, in WO 99/54440 or by Schlereth et al. (Cancer Immunol. Immunother. 20 (2005), 1-12). "Efficacy" or "in vivo efficacy" as used herein refers to the response to therapy by the pharmaceutical composition of the invention, using e.g. standardized NCI response criteria. The success or in vivo efficacy of the therapy using a pharmaceutical composition of the invention refers to the effectiveness of the composition for its intended purpose, i.e. the ability of the composition to cause its desired effect, i.e. depletion of pathologic cells, e.g. tumor cells. The in vivo efficacy may be monitored by established standard methods for the respective disease entities including, but not limited to white blood cell counts, differentials, Fluorescence Activated Cell Sorting, bone marrow aspiration. In addition, various disease specific clinical chemistry parameters and other established standard methods may be used. Furthermore, computer-aided tomography, X-ray, nuclear magnetic resonance tomography (e.g. for National Cancer Institute-criteria based response assessment [Cheson BD, Horning SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, Lister TA, Vose J, Grillo-Lopez A, Hagenbeek A, Cabanillas F, Klippensten D, Hiddemann W, Castellino R, Harris NL, Armitage JO, Carter W, Hoppe R, Canellos GP. Report of an international workshop to standardize response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol. 1999 Apr;17(4):1244]), positron-emission tomography scanning, white blood cell counts, differentials, Fluorescence Activated Cell Sorting, bone marrow aspiration, lymph node biopsies/histologies, and various lymphoma specific clinical chemistry parameters (e.g. lactate dehydrogenase) and other established standard methods may be used.

[252] Another major challenge in the development of drugs such as the pharmaceutical composition of the invention is the predictable modulation of pharmacokinetic properties. To this end, a pharmacokinetic profile of the drug candidate, i.e. a profile of the pharmacokinetic parameters that affect the ability of a particular drug to treat a given condition, can be established. Pharmacokinetic parameters of the drug influencing the ability of a drug for treating a certain disease entity include, but are not limited to: half-life, volume of distribution, hepatic first-pass metabolism and the degree of blood serum binding. The efficacy of a given drug agent can be influenced by each of the parameters mentioned above. It is an

envisaged characteristic of the antigen-binding molecules of the present invention provided with the specific FC modality that they comprise, for example, differences in pharmacokinetic behavior. A half-life extended targeting antigen-binding molecule according to the present invention preferably shows a surprisingly increased residence time in vivo in comparison to "canonical" non-HLE versions of said antigen-binding molecule.

[253] "Half-life" means the time where 50% of an administered drug are eliminated through biological processes, e.g. metabolism, excretion, etc. By "hepatic first-pass metabolism" is meant the propensity of a drug to be metabolized upon first contact with the liver, i.e. during its first pass through the liver. "Volume of distribution" means the degree of retention of a drug throughout the various compartments of the body, like e.g. intracellular and extracellular spaces, tissues and organs, etc. and the distribution of the drug within these compartments. "Degree of blood serum binding" means the propensity of a drug to interact with and bind to blood serum proteins, such as albumin, leading to a reduction or loss of biological activity of the drug.

[254] Pharmacokinetic parameters also include bioavailability, lag time (Tlag), Tmax, absorption rates, more onset and/or Cmax for a given amount of drug administered. "Bioavailability" means the amount of a drug in the blood compartment. "Lag time" means the time delay between the administration of the drug and its detection and measurability in blood or plasma. "Tmax" is the time after which maximal blood concentration of the drug is reached, and "Cmax" is the blood concentration maximally obtained with a given drug. The time to reach a blood or tissue concentration of the drug which is required for its biological effect is influenced by all parameters. Pharmacokinetic parameters of bispecific antigen-binding molecules exhibiting cross-species specificity, which may be determined in preclinical animal testing in non-chimpanzee primates as outlined above, are also set forth e.g. in the publication by Schlereth et al. (Cancer Immunol. Immunother. 20 (2005), 1-12).

[255] In a preferred aspect of the invention the pharmaceutical composition is stable for at least four weeks at about -20°C. As apparent from the appended examples the quality of an antigen-binding molecule of the invention vs. the quality of corresponding state of the art antigen-binding molecules may be tested using different systems. Those tests are understood to be in line with the "ICH Harmonised Tripartite Guideline: *Stability Testing of Biotechnological/Biological Products Q5C* and *Specifications: Test procedures and Acceptance Criteria for Biotech Biotechnological/Biological Products Q6B"* and, thus are elected to provide a stability-indicating profile that provides certainty that changes in the identity, purity and potency of the product are detected. It is well accepted that the term purity is a relative term. Due to the effect of glycosylation, deamidation, or other heterogeneities, the absolute purity of a biotechnological/biological product should be typically assessed by more than one method and the purity value derived is method-dependent. For the purpose of stability testing, tests for purity should focus on methods for determination of degradation products.

[256] For the assessment of the quality of a pharmaceutical composition comprising an antigen-binding molecule of the invention may be analyzed e.g. by analyzing the content of soluble aggregates in a solution (HMWS per size exclusion). It is preferred that stability for at least four weeks at about -20°C is characterized by a content of less than about 5% HMWS, more preferably of less than 2.5% HMWS, even more preferably by less than 1.5% HMWS.

[257] Other examples for the assessment of the stability of an antigen-binding molecule of the invention in form of a pharmaceutical composition are provided in the appended examples 4-12. In those examples embodiments of antigen-binding molecules of the invention are tested with respect to different stress conditions in different pharmaceutical formulations and the results compared with other half-life extending (HLE) formats of bispecific T cell engaging antigen-binding molecule known from the art. In general, it is envisaged that antigen-binding molecules provided with the specific FC modality according to the present invention are typically more stable over a broad range of stress conditions such as temperature and light stress, both compared to antigen-binding molecules provided with different HLE formats and without any HLE format (e.g. "canonical" antigen-binding molecules). Said temperature stability may relate both to decreased (below room temperature including freezing) and increased (above room temperature including temperatures up to or above body temperature) temperature. As the person skilled in the art will acknowledge, such improved stability with regard to stress, which is hardly avoidable in clinical practice, makes the antigen-binding molecule safer because less degradation products will occur in clinical practice. In consequence, said increased stability means increased safety.

[258] One embodiment provides the antigen-binding molecule of the invention or the antigen-binding molecule produced according to the process of the invention for use in the prevention, treatment or amelioration of a proliferative disease, a tumorous disease, a viral disease or an immunological disorder.

[259] The formulations described herein are useful as pharmaceutical compositions in the treatment, amelioration and/or prevention of the pathological medical condition as described herein in a patient in need thereof. The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Treatment includes the application or administration of the formulation to the body, an isolated tissue, or cell from a patient who has a disease/disorder, a symptom of a disease/disorder, or a predisposition toward a disease/disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptom of the disease, or the predisposition toward the disease.

[260] The term "amelioration" as used herein refers to any improvement of the disease state of a patient having a tumor or cancer or a metastatic cancer as specified herein below, by the administration of an antigen-binding molecule according to the invention to a subject in need thereof. Such an improvement may also be seen as a slowing or stopping of the progression of the tumor or cancer or metastatic cancer of the patient. The term "prevention" as used herein means the avoidance of the occurrence or re-

occurrence of a patient having a tumor or cancer or a metastatic cancer as specified herein below, by the administration of an antigen-binding molecule according to the invention to a subject in need thereof.

[261] The term "disease" refers to any condition that would benefit from treatment with the antigen-binding molecule or the pharmaceutic composition described herein. This includes chronic and acute disorders or diseases including those pathological conditions that predispose the mammal to the disease in question.

[262] A "neoplasm" is an abnormal growth of tissue, usually but not always forming a mass. When also forming a mass, it is commonly referred to as a "tumor". Neoplasms or tumors or can be benign, potentially malignant (pre-cancerous), or malignant. Malignant neoplasms are commonly called cancer. They usually invade and destroy the surrounding tissue and may form metastases, i.e., they spread to other parts, tissues or organs of the body. Hence, the term "metatstatic cancer" encompasses metastases to other tissues or organs than the one of the original tumor. Lymphomas and leukemias are lymphoid neoplasms. For the purposes of the present invention, they are also encompassed by the terms "tumor" or "cancer".

[263] The term "viral disease" describes diseases, which are the result of a viral infection of a subject.

[264] The term "immunological disorder" as used herein describes in line with the common definition of this term immunological disorders such as autoimmune diseases, hypersensitivities, immune deficiencies.

[265] In one embodiment the invention provides a method for the treatment or amelioration of a proliferative disease, a tumorous disease, a viral disease or an immunological disorder, comprising the step of administering to a subject in need thereof the antigen-binding molecule of the invention, or produced according to the process of the invention.

[266] The terms "subject in need" or those "in need of treatment" includes those already with the disorder, as well as those in which the disorder is to be prevented. The subject in need or "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[267] The antigen-binding molecule of the invention will generally be designed for specific routes and methods of administration, for specific dosages and frequencies of administration, for specific treatments of specific diseases, with ranges of bio-availability and persistence, among other things. The materials of the composition are preferably formulated in concentrations that are acceptable for the site of administration.

[268] Formulations and compositions thus may be designed in accordance with the invention for delivery by any suitable route of administration. In the context of the present invention, the routes of administration include, but are not limited to

- topical routes (such as epicutaneous, inhalational, nasal, ophthalmic, auricular / aural, vaginal, mucosal);
- enteral routes (such as oral, gastrointestinal, sublingual, sublabial, buccal, rectal); and
- parenteral routes (such as intravenous, intraarterial, intraosseous, intramuscular, intracerebral, intracerebroventricular, epidural, intrathecal, subcutaneous, intraperitoneal, extra-amniotic, intraarticular, intracardiac, intradermal, intralesional, intrauterine, intravesical, intravitreal, transdermal, intranasal, transmucosal, intrasynovial, intraluminal).

[269] The pharmaceutical compositions and the antigen-binding molecule of this invention are particularly useful for parenteral administration, e.g., subcutaneous or intravenous delivery, for example by injection such as bolus injection, or by infusion such as continuous infusion. Pharmaceutical compositions may be administered using a medical device. Examples of medical devices for administering pharmaceutical compositions are described in U.S. Patent Nos. 4,475,196; 4,439,196; 4,447,224; 4,447, 233; 4,486,194; 4,487,603; 4,596,556; 4,790,824; 4,941,880; 5,064,413; 5,312,335; 5,312,335; 5,383,851; and 5,399,163.

[270] In particular, the present invention provides for an uninterrupted administration of the suitable composition. As a non-limiting example, uninterrupted or substantially uninterrupted, i.e. continuous administration may be realized by a small pump system worn by the patient for metering the influx of therapeutic agent into the body of the patient. The pharmaceutical composition comprising the antigen-binding molecule of the invention can be administered by using said pump systems. Such pump systems are generally known in the art, and commonly rely on periodic exchange of cartridges containing the therapeutic agent to be infused. When exchanging the cartridge in such a pump system, a temporary interruption of the otherwise uninterrupted flow of therapeutic agent into the body of the patient may ensue. In such a case, the phase of administration prior to cartridge replacement and the phase of administration following cartridge replacement would still be considered within the meaning of the pharmaceutical means and methods of the invention together make up one "uninterrupted administration" of such therapeutic agent.

[271] The continuous or uninterrupted administration of the antigen-binding molecules of the invention may be intravenous or subcutaneous by way of a fluid delivery device or small pump system including a fluid driving mechanism for driving fluid out of a reservoir and an actuating mechanism for actuating the driving mechanism. Pump systems for subcutaneous administration may include a needle or a cannula for penetrating the skin of a patient and delivering the suitable composition into the patient's body. Said pump systems may be directly fixed or attached to the skin of the patient independently of a vein, artery or blood vessel, thereby allowing a direct contact between the pump system and the skin of the patient. The pump system can be attached to the skin of the patient for 24 hours up to several days. The pump system may be

of small size with a reservoir for small volumes. As a non-limiting example, the volume of the reservoir for the suitable pharmaceutical composition to be administered can be between 0.1 and 50 ml.

[272] The continuous administration may also be transdermal by way of a patch worn on the skin and replaced at intervals. One of skill in the art is aware of patch systems for drug delivery suitable for this purpose. It is of note that transdermal administration is especially amenable to uninterrupted administration, as exchange of a first exhausted patch can advantageously be accomplished simultaneously with the placement of a new, second patch, for example on the surface of the skin immediately adjacent to the first exhausted patch and immediately prior to removal of the first exhausted patch. Issues of flow interruption or power cell failure do not arise.

[273] If the pharmaceutical composition has been lyophilized, the lyophilized material is first reconstituted in an appropriate liquid prior to administration. The lyophilized material may be reconstituted in, e.g., bacteriostatic water for injection (BWFI), physiological saline, phosphate buffered saline (PBS), or the same formulation the protein had been in prior to lyophilisation.

[274] The compositions of the present invention can be administered to the subject at a suitable dose which can be determined e.g. by dose escalating studies by administration of increasing doses of the antigen-binding molecule of the invention exhibiting cross-species specificity described herein to non-chimpanzee primates, for instance macaques. As set forth above, the antigen-binding molecule of the invention exhibiting cross-species specificity described herein can be advantageously used in identical form in preclinical testing in non-chimpanzee primates and as drug in humans. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently.

[275] The term "effective dose" or "effective dosage" is defined as an amount sufficient to achieve or at least partially achieve the desired effect. The term "therapeutically effective dose" is defined as an amount sufficient to cure or at least partially arrest the disease and its complications in a patient already suffering from the disease. Amounts or doses effective for this use will depend on the condition to be treated (the indication), the delivered antigen-binding molecule, the therapeutic context and objectives, the severity of the disease, prior therapy, the patient's clinical history and response to the therapeutic agent, the route of administration, the size (body weight, body surface or organ size) and/or condition (the age and general health) of the patient, and the general state of the patient's own immune system. The proper dose can be adjusted according to the judgment of the attending physician such that it can be administered to the patient once or over a series of administrations, and in order to obtain the optimal therapeutic effect.

[276] A typical dosage may range from about 0.1 μg/kg to up to about 30 mg/kg or more, depending on the factors mentioned above. In specific embodiments, the dosage may range from 1.0 μg/kg up to about 20 mg/kg, optionally from 10 μg/kg up to about 10 mg/kg or from 100 μg/kg up to about 5 mg/kg.

[277] A therapeutic effective amount of an antigen-binding molecule of the invention preferably results in a decrease in severity of disease symptoms, an increase in frequency or duration of disease symptom-free periods or a prevention of impairment or disability due to the disease affliction. For treating target cell antigen-expressing tumors, a therapeutically effective amount of the antigen-binding molecule of the invention, e.g. an anti-target cell antigen/anti-CD3 antigen-binding molecule, preferably inhibits cell growth or tumor growth by at least about 20%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, or at least about 90% relative to untreated patients. The ability of a compound to inhibit tumor growth may be evaluated in an animal model predictive of efficacy

[278] The pharmaceutical composition can be administered as a sole therapeutic or in combination with additional therapies such as anti-cancer therapies as needed, e.g. other proteinaceous and non-proteinaceous drugs. These drugs may be administered simultaneously with the composition comprising the antigen-binding molecule of the invention as defined herein or separately before or after administration of said antigen-binding molecule in timely defined intervals and doses.

[279] The term "effective and non-toxic dose" as used herein refers to a tolerable dose of an inventive antigen-binding molecule which is high enough to cause depletion of pathologic cells, tumor elimination, tumor shrinkage or stabilization of disease without or essentially without major toxic effects. Such effective and non-toxic doses may be determined e.g. by dose escalation studies described in the art and should be below the dose inducing severe adverse side events (dose limiting toxicity, DLT).

[280] The term "toxicity" as used herein refers to the toxic effects of a drug manifested in adverse events or severe adverse events. These side events might refer to a lack of tolerability of the drug in general and/or a lack of local tolerance after administration. Toxicity could also include teratogenic or carcinogenic effects caused by the drug.

[281] The term "safety", "in vivo safety" or "tolerability" as used herein defines the administration of a drug without inducing severe adverse events directly after administration (local tolerance) and during a longer period of application of the drug. "Safety", "in vivo safety" or "tolerability" can be evaluated e.g. at regular intervals during the treatment and follow-up period. Measurements include clinical evaluation, e.g. organ manifestations, and screening of laboratory abnormalities. Clinical evaluation may be carried out and deviations to normal findings recorded/coded according to NCI-CTC and/or MedDRA standards. Organ manifestations may include criteria such as allergy/immunology, blood/bone marrow, cardiac arrhythmia, coagulation and the like, as set forth e.g. in the Common Terminology Criteria for adverse events v3.0 (CTCAE). Laboratory parameters which may be tested include for instance hematology,

clinical chemistry, coagulation profile and urine analysis and examination of other body fluids such as serum, plasma, lymphoid or spinal fluid, liquor and the like. Safety can thus be assessed e.g. by physical examination, imaging techniques (i.e. ultrasound, x-ray, CT scans, Magnetic Resonance Imaging (MRI), other measures with technical devices (i.e. electrocardiogram), vital signs, by measuring laboratory parameters and recording adverse events. For example, adverse events in non-chimpanzee primates in the uses and methods according to the invention may be examined by histopathological and/or histochemical methods.

[282] The above terms are also referred to e.g. in the Preclinical safety evaluation of biotechnology-derived pharmaceuticals S6; ICH Harmonised Tripartite Guideline; ICH Steering Committee meeting on July 16, 1997.

[283] Finally, the invention provides a kit comprising an antigen-binding molecule of the invention or produced according to the process of the invention, a pharmaceutical composition of the invention, a polynucleotide of the invention, a vector of the invention and/or a host cell of the invention.

[284] In the context of the present invention, the term "kit" means two or more components – one of which corresponding to the antigen-binding molecule, the pharmaceutical composition, the vector or the host cell of the invention – packaged together in a container, recipient or otherwise. A kit can hence be described as a set of products and/or utensils that are sufficient to achieve a certain goal, which can be marketed as a single unit.

[285] The kit may comprise one or more recipients (such as vials, ampoules, containers, syringes, bottles, bags) of any appropriate shape, size and material (preferably waterproof, *e.g.* plastic or glass) containing the antigen-binding molecule or the pharmaceutical composition of the present invention in an appropriate dosage for administration (see above). The kit may additionally contain directions for use (*e.g.* in the form of a leaflet or instruction manual), means for administering the antigen-binding molecule of the present invention such as a syringe, pump, infuser or the like, means for reconstituting the antigen-binding molecule of the invention and/or means for diluting the antigen-binding molecule of the invention.

[286] The invention also provides kits for a single-dose administration unit. The kit of the invention may also contain a first recipient comprising a dried / lyophilized antigen-binding molecule and a second recipient comprising an aqueous formulation. In certain embodiments of this invention, kits containing single-chambered and multi-chambered pre-filled syringes (*e.g.*, liquid syringes and lyosyringes) are provided.

[287] It is noted that as used herein, the singular forms "a", "an", and "the", include plural references unless the context clearly indicates otherwise. Thus, for example, reference to "a reagent" includes one or more of such different reagents and reference to "the method" includes reference to equivalent steps and

methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

[288] Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the present invention.

[289] The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

[290] The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range. It includes, however, also the concrete number, e.g., about 20 includes 20.

[291] The term "less than" or "greater than" includes the concrete number. For example, less than 20 means less than or equal to. Similarly, more than or greater than means more than or equal to, or greater than or equal to, respectively.

[292] Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term "comprising" can be substituted with the term "containing" or "including" or sometimes when used herein with the term "having".

[293] When used herein "consisting of" excludes any element, step, or ingredient not specified in the claim element. When used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim.

[294] In each instance herein any of the terms "comprising", "consisting essentially of" and "consisting of" may be replaced with either of the other two terms.

[295] It should be understood that this invention is not limited to the particular methodology, protocols, material, reagents, and substances, etc., described herein and as such can vary. The terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention, which is defined solely by the claims.

[296] All publications and patents cited throughout the text of this specification (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

[297] A better understanding of the present invention and of its advantages will be obtained from the following examples, offered for illustrative purposes only. The examples are not intended to limit the scope of the present invention in any way.

[298] Example 1: Determining impact of protein concentration and pH on BCMAxCD3, DLL3xCD3 and MUC17xCD3 BiTE® antigen-binding molecule stability

Materials and methods for all experiments: Three Half-Life Extended (HLE) BiTE® molecules BCMAxCD3 (SEQ ID NO: 136), DLL3xCD3 (SEQ ID NO: 104) and MUC17xCD3 (SEQ ID NO: 213) according to the invention, were prepared at high concentration ($\geq$ 15 mg/mL) in 24 formulations at three distinct pH values of 4.4, 4.6, 5.2 through a plate-based buffer exchange system, Freeslate Core Module 3 (CM3) liquid handler (Unchained Labs, Pleasanton, CA). Micro-buffer exchange filtration was performed in a pressure chamber and the liquid volume in each well was measured by a VolumeCheck reader (BioMicroLab, Concord, CA), with more details outlined in the paper1. Formulation stock solutions used in all the test were prepared according to commonly known standards. After buffer exchange was completed, the pH and concentration were measured to ensure it within the target values. After the preparation, samples were aliquoted into 96 well plates, sealed and then stored under -30 °C, 2-8 °C and 40 °C for up to 4 weeks. Afterwards, the stability of the samples, in terms of % HMW, were assessed by size exclusion chromatography (SEC) on an ACQUITY UPLC system (Waters, Milford, MA). Samples were injected onto a Waters BEH200 column with a mobile phase containing 250 mM NaCl at pH 6.5. The peak areas for high molecular weight (HMW) and monomer or main peak (MP) were calculated as a percentage of total peak area.

[299] First, the impact of protein concentration and pH was determined on DLL3xCD3 bispecific antigen-binding molecule. As it can be seen from Fig. 1 (results shown under figure capture), %HMW increases significantly with increasing concentration and pH if no additional stabilizing agent is present in the pharmaceutical composition. In detail, at pH 4.4, as protein concentration increases from 4.5 mg/mL to 30.1 mg/mL, %HMW increases from 0.33 % to 7.97%. At a given concentration of 30.1 mg/ml, %HMW increases with increasing pH, i.e. from pH 4.4 from 5.2, %HMW increase from 3.17 % to 17.26%. Accordingly, the %HMW increase more pronouncedly at higher concentration, because from pH 4.4 to 5.2, %HMW increases only from 0.33 % to 2.17% at a protein concentration of 4.5 mg/mL, while %HMW increases from 7.97 % to 17.26% at 30.1 mg/mL. Hence, higher protein concentration formulations require even more stabilization at higher pH values.

[300] Example 2: Determining impact of protein concentration, pH and various excipients on BCMAxCD3, CD33xCD3 and DLL3xCD3 BiTE® antigen-binding molecule stability

As shown in Fig. 2, increasing pH decreases %LMW but conversely increases %HMW which is in line with the finding of Example 1, i.e. for all of representative BCMAxCD3 bispecific antigen-binding molecule (squares; SEQ ID NO: 136), CD33xCD3 bispecific antigen-binding molecule (circles, SEQ ID

NO: 41), and DLL3xCD3 bispecific antigen-binding molecule (triangles, SEQ ID NO: 104). As shown in Fig. 3, %LMW decreases with pH and is independent of concentration. %LMW decreases from over 8% to lower than 5% over the pH range tested. The range of excipients tested, namely amino acids, cyclodextrin and benzyl alcohol did not show significant effect on %LMW at least at the higher bispecific antigen-binding molecule concentration of 20 mg/ml as an illustrative value for a high concentration formulation according to the present invention.

[301] Example 3: Determining impact of stabilizing excipient EDTA versus pH and various excipients on BCMAxCD3 and DLL3xCD3 BiTE® antigen-binding molecule stability

[302] As shown in Fig. 4, EDTA keeps %HMW low over the course of 4 weeks in liquid formulation of about 27 mg/ml. Hence, EDTA and related other chelating agents are identified as excipients for significantly reducing %HMW in bispecific antigen-binding molecules. In detail, molecules of the invention can have significantly higher % HMW at high concentration, e.g. about 12 % in a composition comprising sucrose and glutamic acid at a pH of 5.2 without a further stabilizing agent (see G52Su in Fig. 4A) at a concentration of 27 mg/ml for BCMAxCD3 bispecific antigen-binding molecule (SEQ ID NO: 41). Therein, EDTA mitigates the %HMW under all the testing conditions as compared to the controls at time 0 or time 4 weeks (at -30 °C, 4 °C or 40 °C), e.g. only about 2 % after 4 weeks at 4 °C or -30 °C for BCMAxCD3 molecule at a concentration of 27 mg/mL with the pH in the range of 4.3 to 5.2. ArgHCl has been identified as significantly increasing the %HMW, e.g. over 20% after 4weeks at 4 °C, which shows that the selection of EDTA was not an arbitrary one as it was not foreseeable that Arg would act in a destabilizing way, provoking additional aggregation while EDTA did not. HpbCD mildly decreases the %HMW relative to control, Phe appears to be less effective; while Pro, Trp and benzyl alcohol did not show significant reducing effects on %HMW at the given high molecule concentration of 27 mg/ml. Comparable results were obtained for DLL3xCD3 bispecific antigen-binding molecule (SEQ ID NO: 104)

[303] Example 4: Impact of stabilizing agent EDTA concentration on the stabilization of high concentration formulation of MUC17xCD3 bispecific antigen-binding molecule

As shown in Fig. 5, MUC17xCD3 molecule (SEQ ID NO: 213) without additional stabilizing agent ("G4Su") exhibits significantly higher % HMW at higher pH, i.e. about 6-7% at pH 5 vs. about 1-2% at pH 4.2. Arg HCl significantly increases the % HMW, i.e. over about 10 %at all conditions. HpbCD, Phe and Pro are less effective as compared to stabilizing agent EDTA, with similar level of %HMW as control. Stabilizing agent EDTA mitigates the %HMW under all testing concentrations, i.e. 0.01%, 0.04% and 0.16% (w/v)) as compared to other excipients at time 0 or 4 weeks. Especially at slightly higher pH 4.6 or 5.0, i.e. only about 2 to 3 %HMW are found after 4 weeks at 4°C or -30 °C in a pharmaceutical composition comprising EDTA at any of the three rested concentrations, while those without stabilizing agent exhibited close or over 5%HMW.

Table 5: Sequence table

1.	CD19 VL CDR1	artificial	aa	KASQSVDYDGDSYLN
2.	CD19 VL CDR2	artificial	aa	DASNLVS
3.	CD19 VL CDR3	artificial	aa	QQSTEDPWT
4.	CD19 VH CDR1	artificial	aa	SYWMN
5.	CD19 VH CDR2	artificial	aa	QIWPGDGDTNYNGKFKG
6.	CD19 VH CDR3	artificial	aa	RETTTVGRYYYAMDY
7.	CD19 VL	artificial	aa	DIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYLNWY QQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHP VEKVDAATYHCQQSTEDPWTFGGGTKLEIK
8.	CD19 VH	artificial	aa	QVQLQQSGAELVRPGSSVKISCKASGYAFSSYWMNWVK QRPGQGLEWIGQIWPGDGDTNYNGKFKGKATLTADESS STAYMQLSSLASEDSAVYFCARRETTTVGRYYYAMDYWG QGTTVTVSS
9.	CD3 VH CDR1	artificial	aa	RYTMH
10.	CD3 VH CDR2	artificial	aa	YINPSRGYTNYNQKFKD
11.	CD3 VH CDR3	artificial	aa	YYDDHYCLDY
12.	CD3 VL CDR1	artificial	aa	RASSSVSYMN
13.	CD3 VL CDR2	artificial	aa	DTSKVAS
14.	CD3 VL CDR3	artificial	aa	QQWSSNPLT
15.	CD3 VH	artificial	aa	DIKLQQSGAELARPGASVKMSCKTSGYTFTRYTMHWVK QRPGQGL EWIGYINPSRGYTNYNQKFKDKATLTTDKSSSTAYMQLSS LTSEDSAVYYCARYYDDHYCLDYWGQGTTLTVSS
16.	CD3 VL	artificial	aa	VDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNWYQ QKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTISSM EAEDAATYYCQQWSSNPLTFGAGTKLELK
17.	CD19xCD3 scFv incl linker and his-tag	artificial	aa	DIQLTQSPASLAVSLGQRATISCKASQSVDYDGDSYLNWY QQIPGQPPKLLIYDASNLVSGIPPRFSGSGSGTDFTLNIHP VEKVDAATYHCQQSTEDPWTFGGGTKLEIKGGGGSGGG GSGGGGSQVQLQQSGAELVRPGSSVKISCKASGYAFSSY WMNWVKQRPGQGLEWIGQIWPGDGDTNYNGKFKGK ATLTADESSSTAYMQLSSLASEDSAVYFCARRETTTVGRYY YAMDYWGQGTTVTVSSGGGGSDIKLQQSGAELARPGAS VKMSCKTSGYTFTRYTMHWVKQRPGQGLEWIGYINPSR GYTNYNQKFKDKATLTTDKSSSTAYMQLSSLTSEDSAVYY CARYYDDHYCLDYWGQGTTLTVSSVEGGSGGSGGSGGS GGVDDIQLTQSPAIMSASPGEKVTMTCRASSSVSYMNW

				YQQKSGTSPKRWIYDTSKVASGVPYRFSGSGSGTSYSLTIS SMEAEDAATYYCQQWSSNPLTFGAGTKLELKHHHHHH
18.	CDR-L1 of I2C	artificial	aa	GSSTGAVTSGNYPN
19.	CDR-L2 of I2C	artificial	aa	GTKFLAP
20.	CDR-L3 of I2C	artificial	aa	VLWYSNRWV
21.	CDR-H1 of I2C	artificial	aa	KYAMN
22.	CDR-H2 of I2C	artificial	aa	RIRSKYNNYATYYADSVKD
23.	CDR-H3 of I2C	artificial	aa	HGNFGNSYISYWAY
24.	VH of I2C	artificial	aa	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWV RQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRD DSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYW AYWGQGTLVTVSS
25.	VL of I2C	artificial	aa	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWV QQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
26.	VH-VL of I2C	artificial	aa	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWV RQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRD DSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYW AYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEP SLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQA PRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDE AEYYCVLWYSNRWVFGGGTKLTVL
27.	CD33 ccVH of E11	artificial	aa	QVQLVQSGAEVKKPGESVKVSCKASGYTFTNYGMNWV KQAPGQCLEWMGWINTYTGEPTYADKFQGRVTMTTD TSTSTAYMEIRNLGGDDTAVYYCARWSWSDGYYVYFD YWGQGTSVTVSS
28.	CD33 VH of E11	Artificial	aa	QVQLVQSGAEVKKPGESVKVSCKASGYTFTNYGMNWV KQAPGQGLEWMGWINTYTGEPTYADKFQGRVTMTTD TSTSTAYMEIRNLGGDDTAVYYCARWSWSDGYYVYFD YWGQGTSVTVSS
29.	CD33 HCDR1 of E11	artificial	aa	NYGMN
30.	CD33 HCDR2 of E11	artificial	aa	WINTYTGEPTYADKFQG
31.	CD33 HCDR3 of E11	artificial	aa	WSWSDGYYVYFDY
32.	CD33 CC VL of E11	artificial	aa	DIVMTQSPDSLTVSLGERTTINCKSSQSVLDSSTNKNSLA WYQQKPGQPPKLLLSWASTRESGIPDRFSGSGSGTDFT LTIDSPQPEDSATYYCQQSAHFPITFGCGTRLEIK

33.	CD33 VL of E11	artificial	aa	DIVMTQSPDSLTVSLGERTTINCKSSQSVLDSSTNKNSLA WYQQKPGQPPKLLLSWASTRESGIPDRFSGSGSGTDFT LTIDSPQPEDSATYYCQQSAHFPITFGQGTRLEIK
34.	CD33 LCDR1 of E11	artificial	aa	KSSQSVLDSSTNKNSLA
35.	CD33 LCDR2 of E11	artificial	aa	WASTRES
36.	CD33 LCDR3 of E11	artificial	aa	QQSAHFPIT
37.	CD33 HL CC of E11	artificial	aa	QVQLVQSGAEVKKPGESVKVSCKASGYTFTNYGMNWV KQAPGQCLEWMGWINTYTGEPTYADKFQGRVTMTTD TSTSTAYMEIRNLGGDDTAVYYCARWSWSDGYYVYFD YWGQGTSVTVSSggggsggggsggggsDIVMTQSPDSLTV SLGERTTINCKSSQSVLDSSTNKNSLAWYQQKPGQPPKL LLSWASTRESGIPDRFSGSGSGTDFTLTIDSPQPEDSATY YCQQSAHFPITFGCGTRLEIK
38.	CD33 HL of E11	artificial	aa	QVQLVQSGAEVKKPGESVKVSCKASGYTFTNYGMNWV KQAPGQGLEWMGWINTYTGEPTYADKFQGRVTMTTD TSTSTAYMEIRNLGGDDTAVYYCARWSWSDGYYVYFD YWGQGTSVTVSSGGGGSGGGGSGGGGSDIVMTQSPD SLTVSLGERTTINCKSSQSVLDSSTNKNSLAWYQQKPGQ PPKLLLSWASTRESGIPDRFSGSGSGTDFTLTIDSPQPED SATYYCQQSAHFPITFGQGTRLEIK
39.	CD33 CC E11 HL x I2C HL Bispecific molecule	artificial	aa	QVQLVQSGAEVKKPGESVKVSCKASGYTFTNYGMNWV KQAPGQCLEWMGWINTYTGEPTYADKFQGRVTMTTD TSTSTAYMEIRNLGGDDTAVYYCARWSWSDGYYVYFD YWGQGTSVTVSSGGGGSGGGGSGGGGSDIVMTQSPD SLTVSLGERTTINCKSSQSVLDSSTNKNSLAWYQQKPGQ PPKLLLSWASTRESGIPDRFSGSGSGTDFTLTIDSPQPED SATYYCQQSAHFPITFGCGTRLEIKSGGGGSEVQLVESG GGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGL EWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQ MNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTL VTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGT VTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTK FLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLW YSNRWVFGGGTKLTVL
40.	CD33 E11 HL x I2C HL	artificial	aa	MGWSCIILFLVATATGVHSQVQLVQSGAEVKKPGESVK VSCKASGYTFTNYGMNWVKQAPGQGLEWMGWINTY TGEPTYADKFQGRVTMTTDTSTSTAYMEIRNLGGDDTA VYYCARWSWSDGYYVYFDYWGQGTSVTVSSGGGGSG GGGSGGGGSDIVMTQSPDSLTVSLGERTTINCKSSQSVL DSSTNKNSLAWYQQKPGQPPKLLLSWASTRESGIPDRF SGSGSGTDFTLTIDSPQPEDSATYYCQQSAHFPITFGQG TRLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGF

				TFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYA DSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGG GGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYP NWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGK AALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLH HHHHH
41.	CD33 CC x I2C-scFc Bispecific HLE molecule	artificial	aa	QVQLVQSGAEVKKPGESVKVSCKASGYTFTNYGMNWVK QAPGQCLEWMGWINTYTGEPTYADKFQGRVTMTTDTS TSTAYMEIRNLGGDDTAVYYCARWSWSDGYYVYFDYW GQGTSVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLTV SLGERTTINCKSSQSVLDSSTNKNSLAWYQQKPGQPPKLL LSWASTRESGIPDRFSGSGSGTDFTLTIDSPQPEDSATYYC QQSAHFPITFGCGTRLEIKSGGGGSEVQLVESGGGLVQP GGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIR SKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTE DTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGG SGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGA VTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSG SLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTK LTVLGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP CEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE DPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGK
42.	EGFRvIIIxCD3-scFc VH CDR1	artificial	aa	NYGMH
43.	EGFRvIIIxCD3-scFc VH CDR2	artificial	aa	VIWYDGSDKYYADSVRG
44.	EGFRvIIIxCD3-scFc VH CDR3	artificial	aa	DGYDILTGNPRDFDY
45.	EGFRvIIIxCD3-scFc VL CDR1	artificial	aa	RSSQSLVHSDGNTYLS
46.	EGFRvIIIxCD3-scFc VL CDR2	artificial	aa	RISRRFS

47.	EGFRvIIIxCD3-scFc VL CDR3	artificial	aa	MQSTHVPRT
48.	EGFRvIII_CCxCD3-scFc VH	artificial	aa	QVQLVESGGGVVQSGRSLRLSCAASGFTFRNYGMHWVRQAPGKCLEWVAVIWYDGSDKYYADSVRGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGYDILTGNPRDFDYWGQGTLVTVSS
49.	EGFRvIII_CCxCD3-scFc VL	artificial	aa	DTVMTQTPLSSHVTLGQPASISCRSSQSLVHSDGNTYLSWLQQRPGQPPRLLIYRISRRFSGVPDRFSGSGAGTDFTLEISRVEAEDVGVYYCMQSTHVPRTFGCGTKVEIK
50.	EGFRvIII_CCxCD3-scFc scFv	artificial	aa	QVQLVESGGGVVQSGRSLRLSCAASGFTFRNYGMHWVRQAPGKCLEWVAVIWYDGSDKYYADSVRGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGYDILTGNPRDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDTVMTQTPLSSHVTLGQPASISCRSSQSLVHSDGNTYLSWLQQRPGQPPRLLIYRISRRFSGVPDRFSGSGAGTDFTLEISRVEAEDVGVYYCMQSTHVPRTFGCGTKVEIK
51.	EGFRvIII_CCxCD3-scFc Bispecific molecule	artificial	aa	QVQLVESGGGVVQSGRSLRLSCAASGFTFRNYGMHWVRQAPGKCLEWVAVIWYDGSDKYYADSVRGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGYDILTGNPRDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDTVMTQTPLSSHVTLGQPASISCRSSQSLVHSDGNTYLSWLQQRPGQPPRLLIYRISRRFSGVPDRFSGSGAGTDFTLEISRVEAEDVGVYYCMQSTHVPRTFGCGTKVEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
52.	EGFRvIII_CCxCD3-scFc Bispecific HLE molecule	artificial	aa	QVQLVESGGGVVQSGRSLRLSCAASGFTFRNYGMHWVRQAPGKCLEWVAVIWYDGSDKYYADSVRGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARDGYDILTGNPRDFDYWGQGTLVTVSSGGGGSGGGGSGGGGSDTVMTQTPLSSHVTLGQPASISCRSSQSLVHSDGNTYLSWLQQRPGQPPRLLIYRISRRFSGVPDRFSGSGAGTDFTLEISRVEAEDVGVYYCMQSTHVPRTFGCGTKVEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPI

				EKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGF YPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTV DKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGG GGSGGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPC PAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPE NNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
53.	MSLN_5 VH CDR1	artificial	aa	DYYMT
54.	MSLN_5 VH CDR2	artificial	aa	YISSSGSTIYYADSVKG
55.	MSLN_5 VH CDR3	artificial	aa	DRNSHFDY
56.	MSLN_5 VL CDR1	artificial	aa	RASQGINTWLA
57.	MSLN_5 VL CDR2	artificial	aa	GASGLQS
58.	MSLN_5 VL CDR3	artificial	aa	QQAKSFPRT
59.	MSLN _5 VH	artificial	aa	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMTWIR QAPGKGLEWLSYISSSGSTIYYADSVKGRFTISRDNAKNS LFLQMNSLRAEDTAVYYCARDRNSHFDYWGQGTLVTV SS
60.	MSLN_5 VL	artificial	aa	DIQMTQSPSSVSASVGDRVTITCRASQGINTWLAWYQ QKPGKAPKLLIYGASGLQSGVPSRFSGSGSGTDFTLTISS LQPEDFATYYCQQAKSFPRTFGQGTKVEIK
61.	MSLN_5 scFv	artificial	aa	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMTWIR QAPGKGLEWLSYISSSGSTIYYADSVKGRFTISRDNAKNS LFLQMNSLRAEDTAVYYCARDRNSHFDYWGQGTLVTV SSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVT ITCRASQGINTWLAWYQQKPGKAPKLLIYGASGLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKSFPRTF GQGTKVEIK
62.	MSLN_5xI2C0 bispecific molecule	artificial	aa	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMTWIR QAPGKGLEWLSYISSSGSTIYYADSVKGRFTISRDNAKNS LFLQMNSLRAEDTAVYYCARDRNSHFDYWGQGTLVTV SSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVT ITCRASQGINTWLAWYQQKPGKAPKLLIYGASGLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKSFPRTF GQGTKVEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCA

				ASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYA TYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYC VRHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGG SGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSG NYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLG GKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLT VL

63.	MSLN_5xCD3-scFc Bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGGSLRLSCAASGFTFSDYYMTWIR QAPGKGLEWLSYISSSGSTIYYADSVKGRFTISRDNAKNS LFLQMNSLRAEDTAVYYCARDRNSHFDYWGQGTLVTV SSGGGGSGGGGSGGGGSDIQMTQSPSSVSASVGDRVT ITCRASQGINTWLAWYQQKPGKAPKLLIYGASGLQSGV PSRFSGSGSGTDFTLTISSLQPEDFATYYCQQAKSFPRTF GQGTKVEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCA ASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYA TYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYC VRHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGG SGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSG NYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLG GKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLT VLGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTK PCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKAL PAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCL VKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLY SKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSP GKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSDKT HTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTY RCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSR WQQGNVFSCSVMHEALHNHYTQKSLSLSPGK
64.	MSLN_5_CCxCD3-scFc Bispecific HLE molecule	artificial	aa	qvqlvesggglvkpggslrlscaasgftfsdhymswirqapgkclewf syisssggiiyyadsvkgrftisrdnaknslylqmnslraedtavyycar dvgshfdywgqgtlvtvssggggsggggsggggsdiqmtqspssvs asvgdrvtitcrasqdisrwlawyqqkpgkapkllisaasrlqsgvpsr fsgsgsgtdftltisslqpedfaiyycqqaksfprtfgcgtkveiksgggg sevqlvesggglvqpggslklscaasgftfnkyamnwvrqapgkgle wvarirskynnyatyyadsvkdrftisrddskntaylqmnnlktedt avyycvrhgnfgnsyisywaywgqgtlvtvssggggsggggsgggg sqtvvtqepsltvspggtvtltcgsstgavtsgnypnwvqqkpgqap rgliggtkflapgtparfsgsllggkaaltlsgvqpedeaeyycvlwysn rwvfgggtkltvlggggdkthtcppcpapellggpsvflfppkpkdtl misrtpevtcvvvdvshedpevkfnwyvdgvevhnaktkpceeq ygstyrcvsvltvlhqdwlngkeykckvsnkalpapiektiskakgqp repqvytlppsreemtknqvsltclvkgfypsdiavewesngqpen nykttppvldsdgsfflyskltvdksrwqqgnvfscsvmhealhnhy tqkslslspgkggggsggggsggggsggggsggggsggggsdkthtc ppcpapellggpsvflfppkpkdtlmisrtpevtcvvvdvshedpev kfnwyvdgvevhnaktkpceeqygstyrcvsvltvlhqdwlngkey kckvsnkalpapiektiskakgqprepqvytlppsreemtknqvslt clvkgfypsdiavewesngqpennykttppvldsdgsfflyskltvdk srwqqgnvfscsvmhealhnhytqkslslspgk

65.	CDR-H1 of CDH19 65254.007	artificial	aa	SYGMH
66.	CDR-H2 of CDH19 65254.007	artificial	aa	FIWYEGSNKYYAESVKD
67.	CDR-H3 of CDH19 65254.007	artificial	aa	RAGIIGTIGYYYGMDV
68.	CDR-L1 of CDH19 65254.007	artificial	aa	SGDRLGEKYTS
69.	CDR-L2 of CDH19 65254.007	artificial	aa	QDTKRPS
70.	CDR-L3 of CDH19 65254.007	artificial	aa	QAWESSTVV
71.	VH of CDH19 65254.007	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAFIWYEGSNKYYAESVKDRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGMDV WGQGTTVTVSS
72.	VL of CDH19 65254.007	artificial	aa	SYELTQPPSVSVSPGQTASITCSGDRLGEKYTSWYQQRP GQSPLLVIYQDTKRPSGIPERFSGSNSGNTATLTISGTQA MDEADYYCQAWESSTVVFGGGTKLTVLS
73.	VH-VL of CDH19 65254.007	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAFIWYEGSNKYYAESVKDRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGMDV WGQGTTVTVSSGGGGSGGGGSGGGGSSYELTQPPSVS VSPGQTASITCSGDRLGEKYTSWYQQRPGQSPLLVIYQD TKRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYCQA WESSTVVFGGGTKLTVLS
74.	CDH19 65254.007 x I2C	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHWV RQAPGKGLEWVAFIWYEGSNKYYAESVKDRFTISRDNS KNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGMDV WGQGTTVTVSSGGGGSGGGGSGGGGSSYELTQPPSVS VSPGQTASITCSGDRLGEKYTSWYQQRPGQSPLLVIYQD TKRPSGIPERFSGSNSGNTATLTISGTQAMDEADYYCQA WESSTVVFGGGTKLTVLSGGGGSEVQLVESGGGLVQP GGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLK TEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSG GGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCG

				SSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGT PARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRW VFGGGTKLTVLHHHHHH
75.	CDH19 65254.007 x I2C –scFc Bispecific HLE molecule	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHW VRQAPGKGLEWVAFIWYEGSNKYYAESVKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSSYELTQPP SVSVSPGQTASITCSGDRLGEKYTSWYQQRPGQSPLLVI YQDTKRPSGIPERFSGSNSGNTATLTISGTQAMDEADY YCQAWESSTVVFGGGTKLTVLSGGGGSEVQLVESGGG LVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLE WVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQ MNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTL VTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGT VTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTK FLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVL WYSNRWVFGGGTKLTVLGGGGDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQ DWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVY TLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGG SGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYV DGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRE EMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
76.	CDH19 65254.007 x I2C –scFc_delGK Bispecific HLE molecule	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHW VRQAPGKGLEWVAFIWYEGSNKYYAESVKDRFTISRD NSKNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYG MDVWGQGTTVTVSSGGGGSGGGGSGGGGSSYELTQ PPSVSVSPGQTASITCSGDRLGEKYTSWYQQRPGQSPL LVIYQDTKRPSGIPERFSGSNSGNTATLTISGTQAMDEA DYYCQAWESSTVVFGGGTKLTVLSGGGGSEVQLVESG GGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKG LEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYL QMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQG TLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPG GTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIG GTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYC VLWYSNRWVFGGGTKLTVLGGGGDKTHTCPPCPAPE LLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG

				QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGG GSGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
77.	CDH19 65254.007_CC x I2C –scFc VH	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHW VRQAPGKCLEWVAFIWYEGSNKYYAESVKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGM DVWGQGTTVTVSS
78.	CDH19 65254.007_CC x I2C –scFc VL	artificial	aa	SYELTQPPSVSVSPGQTASITCSGDRLGEKYTSWYQQR PGQSPLLVIYQDTKRPSGIPERFSGSNSGNTATLTISGTQ AMDEADYYCQAWESSTVVFGCGTKLTVL
79.	CDH19 65254.007_CC x I2C –scFc scFv	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHW VRQAPGKCLEWVAFIWYEGSNKYYAESVKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSSYELTQPP SVSVSPGQTASITCSGDRLGEKYTSWYQQRPGQSPLLV IYQDTKRPSGIPERFSGSNSGNTATLTISGTQAMDEADY YCQAWESSTVVFGCGTKLTVL
80.	CDH19 65254.007_CC x I2C –scFc Bispecific molecule	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHW VRQAPGKCLEWVAFIWYEGSNKYYAESVKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSSYELTQPP SVSVSPGQTASITCSGDRLGEKYTSWYQQRPGQSPLLV IYQDTKRPSGIPERFSGSNSGNTATLTISGTQAMDEADY YCQAWESSTVVFGCGTKLTVLSGGGGSEVQLVESGGG LVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLE WVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQ MNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTL VTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGG TVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGT KFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVL WYSNRWVFGGGTKLTVL
81.	CDH19 65254.007_CC x I2C –scFc Bispecific HLE molecule	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHW VRQAPGKCLEWVAFIWYEGSNKYYAESVKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSSYELTQPP SVSVSPGQTASITCSGDRLGEKYTSWYQQRPGQSPLLV IYQDTKRPSGIPERFSGSNSGNTATLTISGTQAMDEADY YCQAWESSTVVFGCGTKLTVLSGGGGSEVQLVESGGG LVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLE WVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQ

				MNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTL VTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGG TVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGT KFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVL WYSNRWVFGGGTKLTVLGGGGDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGG GSGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW YVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWL NGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPP SREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV MHEALHNHYTQKSLSLSPGK
82.	CDH19 65254.007_CC x I2C – scFc_delGK Bispecific HLE molecule	artificial	aa	QVQLVESGGGVVQPGGSLRLSCAASGFTFSSYGMHW VRQAPGKCLEWVAFIWYEGSNKYYAESVKDRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARRAGIIGTIGYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSSYELTQPP SVSVSPGQTASITCSGDRLGEKYTSWYQQRPGQSPLLV IYQDTKRPSGIPERFSGSNSGNTATLTISGTQAMDEADY YCQAWESSTVVFGCGTKLTVLSGGGGSEVQLVESGGG LVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLE WVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQ MNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTL VTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGG TVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGT KFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVL WYSNRWVFGGGTKLTVLGGGGDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGS GGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFL FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVD GVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGK EYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTT PPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK

83.	FLT3_7 A8xCD3-scFc VH CDR1	artificial	aa	narmgvs
84.	FLT3_7 A8xCD3-scFc VH CDR2	artificial	aa	hifsndeksystslkn
85.	FLT3_7 A8xCD3-scFc VH CDR3	artificial	aa	ivgygsgwygffdy
86.	FLT3_7 A8xCD3-scFc VL CDR1	artificial	aa	rasqgirndlg
87.	FLT3_7 A8xCD3-scFc VL CDR2	artificial	aa	aastlqs
88.	FLT3_7 A8xCD3-scFc VL CDR3	artificial	aa	lqhnsyplt
89.	FLT3_7 A8xCD3-scFc VH	artificial	aa	QVTLKESGPTLVKPTETLTLTCTLSGFSLNNARMGVSWIR QPPGKCLEWLAHIFSNDEKSYSTSLKNRLTISKDSSKTQVV LTMTNVDPVDTATYYCARIVGYGSGWYGFFDYWGQGTL VTVSS
90.	FLT3_ A8-scFc VL	artificial	aa	DIQMTQSPSSLSASVGDRVTITCRASQGIRNDLGWYQQK PGKAPKRLIYAASTLQSGVPSRFSGSGSGTEFTLTISSLQPE DFATYYCLQHNSYPLTFGCGTKVEIK
91.	FLT3_7 A8xCD3- scFv	artificial	aa	QVTLKESGPTLVKPTETLTLTCTLSGFSLNNARMGVSWIR QPPGKCLEWLAHIFSNDEKSYSTSLKNRLTISKDSSKTQVV LTMTNVDPVDTATYYCARIVGYGSGWYGFFDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASTLQSGV PSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGC GTKVEIK
92.	FLT3_7 A8xCD3 Bispecific molecule	artificial	aa	QVTLKESGPTLVKPTETLTLTCTLSGFSLNNARMGVSWIR QPPGKCLEWLAHIFSNDEKSYSTSLKNRLTISKDSSKTQVV LTMTNVDPVDTATYYCARIVGYGSGWYGFFDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASTLQSGV PSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGC GTKVEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAASG FTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYA DSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHG NFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGGG SQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWV

				QQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
93.	FLT3_7 A8xCD3-scFc Bispecific HLE molecule	artificial	aa	QVTLKESGPTLVKPTETLTLTCTLSGFSLNNARMGVSWIR QPPGKCLEWLAHIFSNDEKSYSTSLKNRLTISKDSSKTQVV LTMTNVDPVDTATYYCARIVGYGSGWYGFFDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCRASQGIRNDLGWYQQKPGKAPKRLIYAASTLQSGV PSRFSGSGSGTEFTLTISSLQPEDFATYYCLQHNSYPLTFGC GTKVEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAASG FTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYA DSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHG NFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGGG SQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWV QQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
94.	VH CDR1 DLL3_1_CC_delGK	artificial	aa	SYYWS
95.	VH CDR2 DLL3_1_CC_delGK	artificial	aa	YVYYSGTTNYNPSLKS
96.	VH CDR3 DLL3_1_CC_delGK	artificial	aa	IAVTGFYFDY
97.	VL CDR1 DLL3_1_CC_delGK	artificial	aa	RASQRVNNNYLA

98.	VL CDR2 DLL3_1_CC_delGK	artificial	aa	GASSRAT
99.	VL CDR3 DLL3_1_CC_delGK	artificial	aa	QQYDRSPLT
100.	VH DLL3_1_CC_delGK	artificial	aa	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQP PGKCLEWIGYVYYSGTTNYNPSLKSRVTISVDTSKNQFSLK LSSVTAADTAVYYCASIAVTGFYFDYWGQGTLVTVSS
101.	VL DLL3_1_CC_delGK	artificial	aa	EIVLTQSPGTLSLSPGERVTLSCRASQRVNNNYLAWYQQ RPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEP EDFAVYYCQQYDRSPLTFGCGTKLEIK
102.	DLL3_1_CC_delGK	artificial	aa	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQP PGKCLEWIGYVYYSGTTNYNPSLKSRVTISVDTSKNQFSLK LSSVTAADTAVYYCASIAVTGFYFDYWGQGTLVTVSSGG GGSGGGGSGGGGSEIVLTQSPGTLSLSPGERVTLSCRASQ RVNNNYLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGS GSGTDFTLTISRLEPEDFAVYYCQQYDRSPLTFGCGTKLEIK
103.	DLL3_1_CCxCD3_delGK Bispecific molecule	artificial	aa	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQP PGKCLEWIGYVYYSGTTNYNPSLKSRVTISVDTSKNQFSLK LSSVTAADTAVYYCASIAVTGFYFDYWGQGTLVTVSSGG GGSGGGGSGGGGSEIVLTQSPGTLSLSPGERVTLSCRASQ RVNNNYLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGS GSGTDFTLTISRLEPEDFAVYYCQQYDRSPLTFGCGTKLEIK SGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYA MNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRF TISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYI SYWAYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVT QEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPG QAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPE DEAEYYCVLWYSNRWVFGGGTKLTVL
104.	DLL3_1_CCxCD3-scFc_delGK Bispecific HLE molecule	artificial	aa	QVQLQESGPGLVKPSETLSLTCTVSGGSISSYYWSWIRQP PGKCLEWIGYVYYSGTTNYNPSLKSRVTISVDTSKNQFSLK LSSVTAADTAVYYCASIAVTGFYFDYWGQGTLVTVSSGG GGSGGGGSGGGGSEIVLTQSPGTLSLSPGERVTLSCRASQ RVNNNYLAWYQQRPGQAPRLLIYGASSRATGIPDRFSGS GSGTDFTLTISRLEPEDFAVYYCQQYDRSPLTFGCGTKLEIK SGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYA MNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRF TISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYI SYWAYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVT QEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPG QAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPE DEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE

				DPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTV LHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSC SVMHEALHNHYTQKSLSLSPGGGGSGGGGSGGGGSGG GGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPK PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK
105.	VH CDR1 CD19 97-G1RE-C2	artificial	aa	SYGMH
106.	VH CDR2 CD19 97-G1RE-C2	artificial	aa	VISYEGSNKYYAESVKG
107.	VH CDR3 CD19 97-G1RE-C2	artificial	aa	DRGTIFGNYGLEV
108.	VH CD19 97-G1RE-C2 CC	artificial	aa	QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYGMHWVR QAPGKCLEWVAVISYEGSNKYYAESVKGRFTISRDNSKNT LYLQMNSLRDEDTAVYYCARDRGTIFGNYGLEVWGQGT TVTVSS
109.	VL CDR1 CD19 97-G1RE-C2	artificial	aa	RSSQSLLHKNAFNYLD
110.	VL CDR2 CD19 97-G1RE-C2	artificial	aa	LGSNRAS
111.	VL CDR3 CD19 97-G1RE-C2	artificial	aa	MQALQTPFT
112.	VL CD19 97-G1RE-C2 CC	artificial	aa	DIVMTQSPLSLPVISGEPASISCRSSQSLLHKNAFNYLDWY LQKPGQSPQLLIYLGSNRASGVPDRFSGSGSGTDFTLKISR VEAEDVGVYYCMQALQTPFTFGCGTKVDIK
113.	CD19 97-G1RE-C2 CC x I2C0	artificial	aa	MDMRVPAQLLGLLLLWLRGARCDIVMTQSPLSLPVISGE PASISCRSSQSLLHKNAFNYLDWYLQKPGQSPQLLIYLGS NRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQA LQTPFTFGCGTKVDIKGGGGSGGGGSGGGGSQVQLVES GGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKCL EWVAVISYEGSNKYYAESVKGRFTISRDNSKNTLYLQMNS

				LRDEDTAVYYCARDRGTIFGNYGLEVWGQGTTVTVSSG GGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAM NWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTIS RDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISY WAYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVTQE PSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQA PRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDE AEYYCVLWYSNRWVFGGGTKLTVL
114.	CD19 97-G1RE-C2 CC x I2C0-scFc	artificial	aa	MDMRVPAQLLGLLLLWLRGARCDIVMTQSPLSLPVISGE PASISCRSSQSLLHKNAFNYLDWYLQKPGQSPQLLIYLGS NRASGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCMQA LQTPFTFGCGTKVDIKGGGGSGGGGSGGGGSQVQLVES GGGVVQPGRSLRLSCAASGFTFSSYGMHWVRQAPGKCL EWVAVISYEGSNKYYAESVKGRFTISRDNSKNTLYLQMNS LRDEDTAVYYCARDRGTIFGNYGLEVWGQGTTVTVSSG GGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAM NWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTIS RDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISY WAYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVTQE PSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQA PRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDE AEYYCVLWYSNRWVFGGGTKLTVLGGGGDKTHTCPPCP APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHED PEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSG GGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPP KPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEV HNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKV SNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV SLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGK
115.	VH CDR1 CDH3 G8A 6-B12	artificial	aa	sypin
116.	VH CDR2 CDH3 G8A 6-B12	artificial	aa	viwtgggtnyassvkg
117.	VH CDR3 CDH3 G8A 6-B12	artificial	aa	srgvydfdgrgamdy

118.	VL CDR1 CDH3 G8A 6-B12	artificial	aa	kssqsllyssnqknyfa
119.	VL CDR2 CDH3 G8A 6-B12	artificial	aa	wastres
120.	VL CDR3 CDH3 G8A 6-B12	artificial	aa	qqyysypyt
121.	VH CDH3 G8A 6-B12	artificial	aa	EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYPINWVRQA PGKGLEWVGVIWTGGGTNYASSVKGRFTISRDNSKNTVY LQMNSLRAEDTAVYYCAKSRGVYDFDGRGAMDYWGQ GTLVTVSS
122.	VL CDH3 G8A 6-B12	artificial	aa	DIVMTQSPDSLAVSLGERATINCKSSQSLLYSSNQKNYFA WYQQKPGQPPKLLIYWASTRESGVPDRFSGSGSGTDFTL TISSLQAEDVAVYYCQQYYSYPYTFGQGTKLEIK
123.	CDH3 G8A 6-B12 scFv	artificial	aa	EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYPINWVRQA PGKGLEWVGVIWTGGGTNYASSVKGRFTISRDNSKNTVY LQMNSLRAEDTAVYYCAKSRGVYDFDGRGAMDYWGQ GTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSL GERATINCKSSQSLLYSSNQKNYFAWYQQKPGQPPKLLIY WASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QYYSYPYTFGQGTKLEIK
124.	CDH3 G8A 6-B12 x I2C0 bispecific molecule	artificial	aa	EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYPINWVRQA PGKGLEWVGVIWTGGGTNYASSVKGRFTISRDNSKNTVY LQMNSLRAEDTAVYYCAKSRGVYDFDGRGAMDYWGQ GTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSL GERATINCKSSQSLLYSSNQKNYFAWYQQKPGQPPKLLIY WASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QYYSYPYTFGQGTKLEIKSGGGGSEVQLVESGGGLVQPG GSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRS KYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTED TAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAV TSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSL LGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKL TVL
125.	CDH3 G8A 6-B12 x I2C0 bispecific molecule HLE	artificial	aa	EVQLLESGGGLVQPGGSLRLSCAASGFSFSSYPINWVRQA PGKGLEWVGVIWTGGGTNYASSVKGRFTISRDNSKNTVY LQMNSLRAEDTAVYYCAKSRGVYDFDGRGAMDYWGQ GTLVTVSSGGGGSGGGGSGGGGSDIVMTQSPDSLAVSL GERATINCKSSQSLLYSSNQKNYFAWYQQKPGQPPKLLIY WASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQ QYYSYPYTFGQGTKLEIKSGGGGSEVQLVESGGGLVQPG GSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRS

				KYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTED TAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGGS GGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAV TSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSL LGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKL TVLGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMI SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP CEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPA PIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKG GGGSGGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH EDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLT VLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK
126.	BCMA A7 27-C4-G7 CDR1 VH	artificial	aa	NHIIH
127.	BCMA A7 27-C4-G7 CDR2 VH	artificial	aa	YINPYPGYHAYNEKFQG
128.	BCMA A7 27-C4-G7 CDR3 VH	artificial	aa	DGYYRDTDVLDY
129.	BCMA A7 27-C4-G7 CDR1 VL	artificial	aa	QASQDISNYLN
130.	BCMA A7 27-C4-G7 CDR2 VL	artificial	aa	YTSRLHT
131.	BCMA A7 27-C4-G7 CDR3 VL	artificial	aa	QQGNTLPWT
132.	BCMA A7 27-C4-G7 CC (44/100) VH	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNHIIHWVRQ APGQCLEWMGYINPYPGYHAYNEKFQGRATMTSDTSTS TVYMELSSLRSEDTAVYYCARDGYYRDTDVLDYWGQGTL VTVSS
133.	BCMA A7 27-C4-G7 CC (44/100) VL	artificial	aa	DIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQK PGKAPKLLIYYTSRLHTGVPSRFSGSGSGTDFTFTISSLEPE DIATYYCQQGNTLPWTFGCGTKLEIK

134.	BCMA A7 27-C4-G7 CC (44/100) scFv	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNHIIHWVRQAPGQCLEWMGYINPYPGYHAYNEKFQGRATMTSDTSTSTVYMELSSLRSEDTAVYYCARDGYYRDTDVLDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYYTSRLHTGVPSRFSGSGSGTDFTFTISSLEPEDIATYYCQQGNTLPWTFGCGTKLEIK
135.	BCMA A7 27-C4-G7 CC (44/100) x I2C0 bispecific molecule	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNHIIHWVRQAPGQCLEWMGYINPYPGYHAYNEKFQGRATMTSDTSTSTVYMELSSLRSEDTAVYYCARDGYYRDTDVLDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYYTSRLHTGVPSRFSGSGSGTDFTFTISSLEPEDIATYYCQQGNTLPWTFGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
136.	BCMA A7 27-C4-G7 CC (44/100) x I2C0-scFc bispecific molecule HLE	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTNHIIHWVRQAPGQCLEWMGYINPYPGYHAYNEKFQGRATMTSDTSTSTVYMELSSLRSEDTAVYYCARDGYYRDTDVLDYWGQGTLVTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCQASQDISNYLNWYQQKPGKAPKLLIYYTSRLHTGVPSRFSGSGSGTDFTFTISSLEPEDIATYYCQQGNTLPWTFGCGTKVEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

137.	PM 76-B10.17 CC VH CDR1	artificial	aa	DYYMY
138.	PM 76-B10.17 CC VH CDR2	artificial	aa	IISDAGYYTYYSDIIKG
139.	PM 76-B10.17 CC VH CDR3	artificial	aa	GFPLLRHGAMDY
140.	PM 76-B10.17 CC VL CDR1	artificial	aa	KASQNVDANVA
141.	PM 76-B10.17 CC VL CDR2	artificial	aa	SASYVYW
142.	PM 76-B10.17 CC VL CDR3	artificial	aa	QQYDQQLIT
143.	PM 76-B10.17 CC VH	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDAGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSS
144.	PM 76-B10.17 CC VL	artificial	aa	DIQMTQSPSSLSASVGDRVTITCKASQNVDANVAWYQQ KPGQAPKSLIYSASYVYWDVPSRFSGSASGTDFTLTISSVQ SEDFATYYCQQYDQQLITFGCGTKLEIK
145.	PM 76-B10.17 CC scFv	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDAGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDANVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIK
146.	PM 76-B10.17 CC x I2C0 bispecific molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDAGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDANVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
147.	PM 76-B10.17 CC x I2C0-scFc bispecific	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDAGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR

	HLE molecule			VTITCKASQNVDANVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
148.	PM 76-B10.17 CC x I2C0-scFc_delGK bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDAGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDANVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK

149.	PM 76-B10.17 CC x I2C0 CC (103/43)-scFc bispecific molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDAGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDANVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
150.	PM 76-B10.17 CC x I2C0 CC (103/43)-scFc bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDAGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDANVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
151.	PM 76-B10.17 CC x I2C0 CC (103/43)-scFc_delGK bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDAGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDANVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH

				GNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
152.	PM 76-B10.11 CC VH CDR1	artificial	aa	DYYMY
153.	PM 76-B10.11 CC VH CDR2	artificial	aa	IISDGGYYTYYSDIIKG
154.	PM 76-B10.11 CC VH CDR3	artificial	aa	GFPLLRHGAMDY
155.	PM 76-B10.11 CC VL CDR1	artificial	aa	KASQNVDTNVA
156.	PM 76-B10.11 CC VL CDR2	artificial	aa	SASYVYW
157.	PM 76-B10.11 CC VL CDR3	artificial	aa	QQYDQQLIT
158.	PM 76-B10.11 CC VH	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSS
159.	PM 76-B10.11 CC VL	artificial	aa	DIQMTQSPSSLSASVGDRVTITCKASQNVDTNVAWYQQ KPGQAPKSLIYSASYVYWDVPSRFSGSASGTDFTLTISSVQ SEDFATYYCQQYDQQLITFGGGTKLEIK
160.	PM 76-B10.11 CC scFv	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGGGTKLEIK

161.	PM 76-B10.11 CC x I2C0 bispecific molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGGGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCA ASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSG GGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYP NWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKA ALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
162.	PM 76-B10.11 CC x I2C0-scFc bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGGGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCA ASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSG GGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYP NWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKA ALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGG GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQY GSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
163.	PM 76-B10.11 CC x I2C0-scFc_delGK bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGGGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCA ASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV

				RHGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSG GGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYP NWVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKA ALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGG GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQY GSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
164.	PM 76-B10.11 CC x I2C0 CC (103/43)-scFc bispecific molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGGGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCA ASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHGNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSG GGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYP NWVQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKA ALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
165.	PM 76-B10.11 CC x I2C0 CC (103/43)-scFc bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGGGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCA ASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHGNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSG GGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYP NWVQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKA ALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGG GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQY GSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS

				RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAP ELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPE VKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLH QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK
166.	PM 76-B10.11 CC x I2C0 CC (103/43)-scFc_delGK bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKGLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGGGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCA ASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCV RHGNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSG GGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYP NWVQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKA ALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGG GGDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQY GSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
167.	PM 76-B10.11 CC x I2C0-scFc VH CDR1	artificial	aa	DYYMY
168.	PM 76-B10.11 CC x I2C0-scFc VH CDR2	artificial	aa	IISDGGYYTYYSDIIKG
169.	PM 76-B10.11 CC x I2C0-scFc VH CDR3	artificial	aa	GFPLLRHGAMDY

170.	PM 76-B10.11 CC x I2C0-scFc VL CDR1	artificial	aa	KASQNVDTNVA
171.	PM 76-B10.11 CC x I2C0-scFc VL CDR2	artificial	aa	SASYVYW
172.	PM 76-B10.11 CC x I2C0-scFc VL CDR3	artificial	aa	QQYDQQLIT
173.	PM 76-B10.11 CC x I2C0-scFc VH	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSS
174.	PM 76-B10.11 CC x I2C0-scFc VL	artificial	aa	DIQMTQSPSSLSASVGDRVTITCKASQNVDTNVAWYQQ KPGQAPKSLIYSASYVYWDVPSRFSGSASGTDFTLTISSVQ SEDFATYYCQQYDQQLITFGCGTKLEIK
175.	PM 76-B10.11 CC x I2C0-scFc scFv	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIK
176.	PM 76-B10.11 CC x I2C0-scFc bispecific molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
177.	PM 76-B10.11 CC x I2C0-scFc bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH

				GNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
178.	PM 76-B10.11 CC x I2C0-scFc_delGK bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
179.	PM 76-B10.11 CC x I2C0 CC (103/43)-scFc	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW

	bispecific molecule			DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
180.	PM 76-B10.11 CC x I2C0 CC (103/43)-scFc bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGGG GSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELL GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNY KTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
181.	PM 76-B10.11 CC x I2C0 CC (103/43)-scFc_delGK bispecific HLE molecule	artificial	aa	QVQLVESGGGLVKPGESLRLSCAASGFTFSDYYMYWVR QAPGKCLEWVAIISDGGYYTYYSDIIKGRFTISRDNAKNSL YLQMNSLKAEDTAVYYCARGFPLLRHGAMDYWGQGTL VTVSSGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDR VTITCKASQNVDTNVAWYQQKPGQAPKSLIYSASYVYW DVPSRFSGSASGTDFTLTISSVQSEDFATYYCQQYDQQLIT FGCGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYCGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNW VQQKPGQCPRGLIGGTKFLAPGTPARFSGSLLGGKAALTL SGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGDK THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVV

				VDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTYR CVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKG QPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGGGGSGGGGSG GGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWY VDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREE MTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPP VLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHN HYTQKSLSLSPGK
182.	IgG1 hinge	artificial	aa	DKTHTCPPCP
183.	IgG2 subtype hinge	artificial	aa	ERKCCVECPPCP
184.	IgG3 subtype hinge	artificial	aa	ELKTPLDTTHTCPRCP
185.	IgG3 subtype hinge	artificial	aa	ELKTPLGDTTHTCPRCP
186.	IgG4 subtype hinge	artificial	aa	ESKYGPPCPSCP
187.	G4S linker	artificial	aa	GGGGS
188.	(G4S)2 linker	artificial	aa	GGGGSGGGGS
189.	(G4S)3 linker	artificial	aa	GGGGSGGGGSGGGGS
190.	(G4S)4 linker	artificial	aa	GGGGSGGGGSGGGGSGGGGS
191.	(G4S)5 linker	artificial	aa	GGGGSGGGGSGGGGSGGGGSGGGGS
192.	(G4S)6 linker	artificial	aa	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
193.	(G4S)7 linker	artificial	aa	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
194.	(G4S)8 linker	artificial	aa	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGS
195.	Peptide linker	artificial	aa	PGGGGS
196.	Peptide linker	artificial	aa	PGGDGS
197.	Peptide linker	artificial	aa	SGGGGS
198.	Peptide linker	artificial	aa	GGGG
199.	hexa-histidine tag	artificial	aa	HHHHHH

200.	CD3e binder VL	artificial	aa	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWV QQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAALTLS GVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
201.	CD3e binder VH	artificial	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFNSYAMNWVR QAPGKGLEWVARIRSKYNNYATYYADSVKGRFTISRDDS KNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWWAY WGQGTLVTVSS
202.	CD3e binder scFv	artificial	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFNSYAMNWVR QAPGKGLEWVARIRSKYNNYATYYADSVKGRFTISRDDS KNTAYLQMNSLKTEDTAVYYCVRHGNFGNSYVSWWAY WGQGTLVTVSSGGGGSGGGGSGGGGSQTVVTQEPSLT VSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRG LIGGTKFLAPGTPARFSGSLLGGKAALTLSGVQPEDEAEY YCVLWYSNRWVFGGGTKLTVL
203.	MU 8-B7 CC x I2C0-scFc VH CDR1	artificial	aa	GYYWS
204.	MU 8-B7 CC x I2C0-scFc VH CDR2	artificial	aa	DIDASGSTKYNPSLKS
205.	MU 8-B7 CC x I2C0-scFc VH CDR3	artificial	aa	KKYSTVWSYFDN
206.	MU 8-B7 CC x I2C0-scFc VL CDR1	artificial	aa	SGDKLGDKYAS
207.	MU 8-B7 CC x I2C0-scFc VL CDR2	artificial	aa	QDRKRPS
208.	MU 8-B7 CC x I2C0-scFc VL CDR3	artificial	aa	QAWGSSTAV
209.	MU 8-B7 CC x I2C0-scFc VH	artificial	aa	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIR QPPGKCLEWIGDIDASGSTKYNPSLKSRVTISLDTSKNQFS LKLNSVTAADTAVYFCARKKYSTVWSYFDNWGQGTLVT VSS
210.	MU 8-B7 CC x I2C0-scFc VL	artificial	aa	SYELTQPSSVSVPPGQTASITCSGDKLGDKYASWYQQKP GQSPVLVIYQDRKRPSGVPERFSGSNSGNTATLTISGTQA MDEADYYCQAWGSSTAVFGCGTKLTVL
211.	MU 8-B7 CC x I2C0-scFc scFv	artificial	aa	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIR QPPGKCLEWIGDIDASGSTKYNPSLKSRVTISLDTSKNQFS LKLNSVTAADTAVYFCARKKYSTVWSYFDNWGQGTLVT

				VSSGGGGSGGGGSGGGGSSYELTQPSSVSVPPGQTASIT CSGDKLGDKYASWYQQKPGQSPVLVIYQDRKRPSGVPE RFSGSNSGNTATLTISGTQAMDEADYYCQAWGSSTAVF GCGTKLTVL
212.	MU 8-B7 CC x I2C0-scFc Bispecific molecule	artificial	aa	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIR QPPGKCLEWIGDIDASGSTKYNPSLKSRVTISLDTSKNQFS LKLNSVTAADTAVYFCARKKYSTVWSYFDNWGQGTLVT VSSGGGGSGGGGSGGGGSSYELTQPSSVSVPPGQTASIT CSGDKLGDKYASWYQQKPGQSPVLVIYQDRKRPSGVPE RFSGSNSGNTATLTISGTQAMDEADYYCQAWGSSTAVF GCGTKLTVLSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATY YADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVR HGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGG GGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPN WVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
213.	MU 8-B7 CC x I2C0-scFc Bispecific HLE molecule	artificial	aa	QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIR QPPGKCLEWIGDIDASGSTKYNPSLKSRVTISLDTSKNQFS LKLNSVTAADTAVYFCARKKYSTVWSYFDNWGQGTLVT VSSGGGGSGGGGSGGGGSSYELTQPSSVSVPPGQTASIT CSGDKLGDKYASWYQQKPGQSPVLVIYQDRKRPSGVPE RFSGSNSGNTATLTISGTQAMDEADYYCQAWGSSTAVF GCGTKLTVLSGGGGSEVQLVESGGGLVQPGGSLKLSCAA SGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATY YADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVR HGNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGG GGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPN WVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGG GDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEV TCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQY GSTYRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPS DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKS RWQQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGS GGGGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPA PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDP EVKFNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVL HQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQV YTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCS VMHEALHNHYTQKSLSLSPGK
214.	CL-1 VH CDR1	artificial	aa	GYYMH
215.	CL-1 VH CDR2	artificial	aa	WINPNSGGTKYAQKFQG

216.	CL-1 VH CDR3	artificial	aa	DRITVAGTYYYYGMDV
217.	CL-1 VL CDR1	artificial	aa	RASQGVNNWLA
218.	CL-1 VL CDR2	artificial	aa	TASSLQS
219.	CL-1 VL CDR3	artificial	aa	QQANSFPIT
220.	CL-1 VH	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWV RQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGMD VWGQGTTVTVSS
221.	CL-1 VL	artificial	aa	DIQMTQSPSSVSASVGDRVTITCRASQGVNNWLAWYQ QKPGKAPKLLIYTASSLQSGVPSRFSGSGSGTDFTLTIRSL QPEDFATYYCQQANSFPITFGCGTRLEIK
222.	CL-1 scFv	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWV RQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGMD VWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPS SVSASVGDRVTITCRASQGVNNWLAWYQQKPGKAPKLL IYTASSLQSGVPSRFSGSGSGTDFTLTIRSLQPEDFATYYC QQANSFPITFGCGTRLEIK
223.	CL-1 x I2C bispecific molecule	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWV RQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGMD VWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPS SVSASVGDRVTITCRASQGVNNWLAWYQQKPGKAPKLL IYTASSLQSGVPSRFSGSGSGTDFTLTIRSLQPEDFATYYC QQANSFPITFGCGTRLEIKSGGGGSEVQLVESGGGLVQP GGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGG GGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSS TGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPAR FSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVL
224.	CL-1 x I2C-6His bispecific molecule -his tag	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWV RQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGMD VWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPS SVSASVGDRVTITCRASQGVNNWLAWYQQKPGKAPKLL IYTASSLQSGVPSRFSGSGSGTDFTLTIRSLQPEDFATYYC QQANSFPITFGCGTRLEIKSGGGGSEVQLVESGGGLVQP GGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGG GGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSS TGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPAR

				FSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVLHHHHHH
225.	CL-1 x I2C-scFc bispecific scFc molecule	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWV RQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGMD VWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSPS SVSASVGDRVTITCRASQGVNNWLAWYQQKPGKAPKLL IYTASSLQSGVPSRFSGSGSGTDFTLTIRSLQPEDFATYYC QQANSFPITFGCGTRLEIKSGGGGSEVQLVESGGGLVQP GGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGG GGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSS TGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPAR FSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVLGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTY RCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
226.	CL-2 VH CDR1	artificial	aa	GYYMH
227.	CL-2 VH CDR2	artificial	aa	WINPNSGGTKYAQKFQG
228.	CL-2 VH CDR3	artificial	aa	DRITVAGTYYYYGMDV
229.	CL-2 VL CDR1	artificial	aa	RASQGVNNWLA
230.	CL-2VL CDR2	artificial	aa	TASSLQS
231.	CL-2 VL CDR3	artificial	aa	QQANSFPIT
232.	CL-2 VH	artificial	aa	QVQMVQSGAEVKKHGASVKVSCKASGYTFTGYYMHW VRQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTR DTSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGM DVWGQGTTVTVSS
233.	CL-2 VL	artificial	aa	DIQMTQSPSSVSASVGDRVTITCRASQGVNNWLAWYQ QKPGKAPKLLIYTASSLQSGVPSRFSGSGSGTDFTLTIRSL QPEDFATYYCQQANSFPITFGCGTRLEIK
234.	CL-2 scFv	artificial	aa	QVQMVQSGAEVKKHGASVKVSCKASGYTFTGYYMHW VRQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTR

				DTSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSP SSVSASVGDRVTITCRASQGVNNWLAWYQQKPGKAPKL LIYTASSLQSGVPSRFSGSGSGTDFTLTIRSLQPEDFATYYC QQANSFPITFGCGTRLEIK
235.	CL-2 x I2C bispecific molecule	artificial	aa	QVQMVQSGAEVKKHGASVKVSCKASGYTFTGYYMHW VRQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTR DTSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSP SSVSASVGDRVTITCRASQGVNNWLAWYQQKPGKAPKL LIYTASSLQSGVPSRFSGSGSGTDFTLTIRSLQPEDFATYYC QQANSFPITFGCGTRLEIKSGGGGSEVQLVESGGGLVQP GGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGG GGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSS TGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPAR FSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVL
236.	CL-2 x I2C-6His bispecific molecule - his tag	artificial	aa	QVQMVQSGAEVKKHGASVKVSCKASGYTFTGYYMHW VRQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTR DTSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSP SSVSASVGDRVTITCRASQGVNNWLAWYQQKPGKAPKL LIYTASSLQSGVPSRFSGSGSGTDFTLTIRSLQPEDFATYYC QQANSFPITFGCGTRLEIKSGGGGSEVQLVESGGGLVQP GGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGG GGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSS TGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPAR FSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVLHHHHHH
237.	CL-2 x I2C-scFc bispecific scFc molecule	artificial	aa	QVQMVQSGAEVKKHGASVKVSCKASGYTFTGYYMHW VRQAPGQCLEWMGWINPNSGGTKYAQKFQGRVTMTR DTSISTAYMELSRLRSDDTAVYYCARDRITVAGTYYYYGM DVWGQGTTVTVSSGGGGSGGGGSGGGGSDIQMTQSP SSVSASVGDRVTITCRASQGVNNWLAWYQQKPGKAPKL LIYTASSLQSGVPSRFSGSGSGTDFTLTIRSLQPEDFATYYC QQANSFPITFGCGTRLEIKSGGGGSEVQLVESGGGLVQP GGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKT EDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSGG GGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGSS TGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPAR FSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVLGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPK

				DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV VVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTY RCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK
238.	CD70 HCDR1	artificial	aa	TYAMS
239.	CD70 HCDR2	artificial	aa	AISGSGGRTFYAESVEG
240.	CD70 HCDR3	artificial	aa	HDYSNYPYFDY
241.	CD70 LCDR1	artificial	aa	RASQSVRSTYLA
242.	CD70 LCDR2	artificial	aa	GASSRAT
243.	CD70 LCDR3	artificial	aa	QQYGDLPFT
244.	CD70 VH	artificial	aa	EVQLLESGGGMVQPGGSLRLSCAASGFTFSTYAMSWVR QAPGKCLEWVSAISGSGGRTFYAESVEGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAKHDYSNYPYFDYWGQGTLV TVSS
245.	CD70 VL	artificial	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVRSTYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYSCQQYGDLPFTFGCGTKLEIK
246.	CD70 VHVL	artificial	aa	EVQLLESGGGMVQPGGSLRLSCAASGFTFSTYAMSWVR QAPGKCLEWVSAISGSGGRTFYAESVEGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAKHDYSNYPYFDYWGQGTLV TVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERAT LSCRASQSVRSTYLAWYQQKPGQAPRLLIYGASSRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYSCQQYGDLPFTFG CGTKLEI
247.	CD70 x I2C	artificial	aa	EVQLLESGGGMVQPGGSLRLSCAASGFTFSTYAMSWVR QAPGKCLEWVSAISGSGGRTFYAESVEGRFTISRDNSKNT LYLQMNSLRAEDTAVYYCAKHDYSNYPYFDYWGQGTLV TVSSGGGGSGGGGSGGGGSEIVLTQSPGTLSLSPGERAT LSCRASQSVRSTYLAWYQQKPGQAPRLLIYGASSRATGIP DRFSGSGSGTDFTLTISRLEPEDFAVYSCQQYGDLPFTFG CGTKLEIKSGGGGSEVQLVESGGGLVQPGGSLKLSCAAS GFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYY ADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRH GNFGNSYISYWAYWGQGTLVTVSSGGGGSGGGGSGGG GSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPN

				WVQQKPGQAPRGLIGGTKFLAPGTPARFSGSLLGGKAA LTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTV
248.	B6L / CDR-H1	artificial	aa	GYYMH
249.	B6L / CDR-H2	artificial	aa	WINPNSGETNYAQKFQG
250.	B6L / CDR-H3	artificial	aa	DALIVVAPVTRDYYYYGMDV
251.	B6L / CDR-L1	artificial	aa	RASQSVSSSYLA
252.	B6L / CDR-L2	artificial	aa	GASSRAT
253.	B6L / CDR-L3	artificial	aa	QQYGSSPLT
254.	B6L / VH	artificial	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMHWV RQAPGQCLEWMGWINPNSGETNYAQKFQGRVTMTRD TSISTAYMELSRLRSDDTAVYYCARDALIVVAPVTRDYYYY GMDVWGQGTTVTVSS
255.	B6L / VL	artificial	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQYGSSPLTFGCGTKLEIK
256.	B6L /SCFV I2E	artificial	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQYGSSPLTFGCGTKLEIKGGGGSGGGGSGG GGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMH WVRQAPGQCLEWMGWINPNSGETNYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARDALIVVAPVTRD YYYYGMDVWGQGTTVTVSS
257.	B6L / BISPECIFIC MOL I2E	artificial	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQYGSSPLTFGCGTKLEIKGGGGSGGGGSGG GGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMH WVRQAPGQCLEWMGWINPNSGETNYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARDALIVVAPVTRD YYYYGMDVWGQGTTVTVSSSGGGGSEVQLVESGGGLV QPGGSLKLSCAASGFTFNKYAINWVRQAPGKGLEWVAR IRSKYNNYATYYADAVKDRFTISRDDSKNTVYLQMNNLK TEDTAVYYCARAGNFGSSYISYWAYWGQGTLVTVSSGG GGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTITCGSST GAVTSGNYPNWVQKKPGQAPRGLIGGTKFLAPGTPARF SGSLSGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGS GTKLTVL
258.	B6L / HLE-BITE I2E	artificial	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQYGSSPLTFGCGTKLEIKGGGGSGGGGSGG GGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMH WVRQAPGQCLEWMGWINPNSGETNYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARDALIVVAPVTRD YYYYGMDVWGQGTTVTVSSSGGGGSLVQPGGSLKLSCA ASGFTFNKYAINWVRQAPGKGLEWVARIRSKYNNYATY YADAVKDRFTISRDDSKNTVYLQMNNLKTEDTAVYYCAR AGNFGSSYISYWAYWGQGTLVTVSSGGGGSGGGGSGG GGSQTVVTQEPSLTVSPGGTVTITCGSSTGAVTSGNYPN WVQKKPGQAPRGLIGGTKFLAPGTPARFSGSLSGGKAAL TLSGVQPEDEAEYYCVLWYSNRWVFGSGTKLTVLGGGG DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGST

				YRCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIA VEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRW QQGNVFSCSVMHEALHNHYTQKSLSLSPGKGGGGSGG GGSGGGGSGGGGSGGGGSGGGGSDKTHTCPPCPAPEL LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVK FNWYVDGVEVHNAKTKPCEEQYGSTYRCVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLP PSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGK
259.	B6L / SCFV I2C	artificial	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQYGSSPLTFGCGTKLEIKGGGGSGGGGSGG GGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMH WVRQAPGQCLEWMGWINPNSGETNYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARDALIVVAPVTRD YYYYGMDVWGQGTTVTVSS
260.	B6L / BISPECIFIC MOL I2C	artificial	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQYGSSPLTFGCGTKLEIKGGGGSGGGGSGG GGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMH WVRQAPGQCLEWMGWINPNSGETNYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARDALIVVAPVTRD YYYYGMDVWGQGTTVTVSSSGGGGSEVQLVESGGGLV QPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNL KTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSG GGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGS STGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPA RFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVL
261.	B6L / HLE BITE I2C	artificial	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQK PGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPE DFAVYYCQQYGSSPLTFGCGTKLEIKGGGGSGGGGSGG GGSQVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMH WVRQAPGQCLEWMGWINPNSGETNYAQKFQGRVTM TRDTSISTAYMELSRLRSDDTAVYYCARDALIVVAPVTRD YYYYGMDVWGQGTTVTVSSSGGGGSEVQLVESGGGLV QPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVA RIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNL KTEDTAVYYCVRHGNFGNSYISYWAYWGQGTLVTVSSG GGGSGGGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCGS STGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPA RFSGSLLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFG GGTKLTVLGGGGDKTHTCPPCPAPELLGGPSVFLFPPKPK DTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPCEEQYGSTYRCVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS LSPGKGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSD KTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCV

				VVDVSHEDPEVKFNWYVDGVEVHNAKTKPCEEQYGSTY RCVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQ QGNVFSCSVMHEALHNHYTQKSLSLSPGK

Claims

1. A pharmaceutical composition which is preferably liquid comprising

(a) a bispecific antigen-binding molecule comprising at least three domains, wherein:

- a first domain binds to a target cell surface antigen, wherein the target cell surface antigen is a tumor antigen;
- a second domain binds to an extracellular epitope of the human and/or the *Macaca* CD3 chain; and
- a third domain comprises two polypeptide monomers, each comprising a hinge, a CH2 domain and a CH3 domain, wherein said two polypeptide monomers are fused to each other via a peptide linker, and wherein said third domain comprises in an amino to carboxyl order:

hinge-CH2-CH3-linker-hinge-CH2-CH3;

wherein the concentration of the bispecific antigen-binding molecule is 8 to 35 mg/ml;

(b) at least one buffer agent;

(c) at least one saccharide; and

(d) at least one stabilizing agent selected from Ethylenediaminetetraacetic acid (EDTA), Diethylenetriaminepentetic acid (DTPA), and citric acid, wherein the stabilizing agent is present in a concentration in the range of 0.005% to 0.25% (w/v), preferably 0.01 to 0.2% (w/v);

and wherein the pH of the pharmaceutical composition is in the range of 4.0 to 6.0.

2. The pharmaceutical composition of claim 1, wherein the bispecific antigen-binding molecule is a single chain molecule.

3. The pharmaceutical composition of claim 1, wherein bispecific antigen-binding molecule is half-life extended.

4. The pharmaceutical composition of claim 1, wherein a glycosylation site at Kabat position 314 of the CH2 domains in the third domain of the bispecific antigen-binding molecule is removed by a N314X substitution, wherein X is any amino acid excluding Q.

5. The pharmaceutical composition of any of claim 1, wherein each of said polypeptide monomers of the third domain has an amino acid sequence that is at least 90% identical to a sequence selected from the group consisting of: SEQ ID NOs: 17-24, or has an amino acid sequence selected from the group consisting of SEQ ID NOs: 17-24.

6. The pharmaceutical composition of any of claim 1, wherein the CH2 domain comprises an intra domain cysteine disulfide bridge.

7. The pharmaceutical composition according to claim 1, wherein the tumor antigen is selected from the group consisting of CDH19, CDH3, MSLN, DLL3, FLT3, EGFRvIII, BCMA, PSMA, CD33, CD19, CD20, CLDN18.2, MUC17, EpCAM, CD70 and CLDN6.

8. The pharmaceutical composition of any of claim 1, wherein the second domain is an extracellular epitope of the human and/or the Macaca CD3ε chain.

9. The pharmaceutical composition of any of claim 1, wherein

(i) the first domain comprises two antibody variable domains and the second domain comprises two antibody variable domains;

(ii) the first domain comprises one antibody variable domain and the second domain comprises two antibody variable domains;

(iii) the first domain comprises two antibody variable domains and the second domain comprises one antibody variable domain; or

(iv) the first domain comprises one antibody variable domain and the second domain comprises one antibody variable domain.

10. The pharmaceutical composition of claim 1, wherein the antibody construct comprises in an amino to carboxyl order:

(a) the first domain;

(b) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 187-189;

(c) the second domain;

(d) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 187, 188, 189, 195, 196, 197 and 198;

(e) the first polypeptide monomer of the third domain;

(f) a peptide linker having an amino acid sequence selected from the group consisting of SEQ ID NOs: 191, 192, 193 and 194; and

(g) the second polypeptide monomer of the third domain.

11. The pharmaceutical composition of claim 1, wherein the first binding domain of the construct comprises a VH region comprising CDR-H1, CDR-H2 and CDR-H3 and a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from the group consisting of:

(a) CDR-H1 as depicted in SEQ ID NO: 4, CDR-H2 as depicted in SEQ ID NO: 5, CDR-H3 as depicted in SEQ ID NO: 6, CDR-L1 as depicted in SEQ ID NO: 1, CDR-L2 as depicted in SEQ ID NO: 2 and CDR-L3 as depicted in SEQ ID NO: 3,

(b) CDR-H1 as depicted in SEQ ID NO: 29, CDR-H2 as depicted in SEQ ID NO: 30, CDR-H3 as depicted in SEQ ID NO: 31, CDR-L1 as depicted in SEQ ID NO: 34, CDR-L2 as depicted in SEQ ID NO: 35 and CDR-L3 as depicted in SEQ ID NO: 36,

(c) CDR-H1 as depicted in SEQ ID NO: 42, CDR-H2 as depicted in SEQ ID NO: 43, CDR-H3 as depicted in SEQ ID NO: 44, CDR-L1 as depicted in SEQ ID NO: 45, CDR-L2 as depicted in SEQ ID NO: 46 and CDR-L3 as depicted in SEQ ID NO: 47,

(d) CDR-H1 as depicted in SEQ ID NO: 53, CDR-H2 as depicted in SEQ ID NO: 54, CDR-H3 as depicted in SEQ ID NO: 55, CDR-L1 as depicted in SEQ ID NO: 56, CDR-L2 as depicted in SEQ ID NO: 57 and CDR-L3 as depicted in SEQ ID NO: 58,

(e) CDR-H1 as depicted in SEQ ID NO: 65, CDR-H2 as depicted in SEQ ID NO: 66, CDR-H3 as depicted in SEQ ID NO: 67, CDR-L1 as depicted in SEQ ID NO: 68, CDR-L2 as depicted in SEQ ID NO: 69 and CDR-L3 as depicted in SEQ ID NO: 70,

(f) CDR-H1 as depicted in SEQ ID NO: 83, CDR-H2 as depicted in SEQ ID NO: 84, CDR-H3 as depicted in SEQ ID NO: 85, CDR-L1 as depicted in SEQ ID NO: 86, CDR-L2 as depicted in SEQ ID NO: 87 and CDR-L3 as depicted in SEQ ID NO: 88,

(g) CDR-H1 as depicted in SEQ ID NO: 94, CDR-H2 as depicted in SEQ ID NO: 95, CDR-H3 as depicted in SEQ ID NO: 96, CDR-L1 as depicted in SEQ ID NO: 97, CDR-L2 as depicted in SEQ ID NO: 98 and CDR-L3 as depicted in SEQ ID NO: 99,

(h) CDR-H1 as depicted in SEQ ID NO: 105, CDR-H2 as depicted in SEQ ID NO: 106, CDR-H3 as depicted in SEQ ID NO: 107, CDR-L1 as depicted in SEQ ID NO: 109, CDR-L2 as depicted in SEQ ID NO: 110 and CDR-L3 as depicted in SEQ ID NO: 111,

(i) CDR-H1 as depicted in SEQ ID NO: 115, CDR-H2 as depicted in SEQ ID NO: 116, CDR-H3 as depicted in SEQ ID NO: 117, CDR-L1 as depicted in SEQ ID NO: 118, CDR-L2 as depicted in SEQ ID NO: 119 and CDR-L3 as depicted in SEQ ID NO: 120,

(j) CDR-H1 as depicted in SEQ ID NO: 126, CDR-H2 as depicted in SEQ ID NO: 127, CDR-H3 as depicted in SEQ ID NO: 128, CDR-L1 as depicted in SEQ ID NO: 129, CDR-L2 as depicted in SEQ ID NO: 130 and CDR-L3 as depicted in SEQ ID NO: 131,

(k) CDR-H1 as depicted in SEQ ID NO: 137, CDR-H2 as depicted in SEQ ID NO: 138, CDR-H3 as depicted in SEQ ID NO: 139, CDR-L1 as depicted in SEQ ID NO: 140, CDR-L2 as depicted in SEQ ID NO: 141 and CDR-L3 as depicted in SEQ ID NO: 142,

(l) CDR-H1 as depicted in SEQ ID NO: 152, CDR-H2 as depicted in SEQ ID NO: 153, CDR-H3 as depicted in SEQ ID NO: 154, CDR-L1 as depicted in SEQ ID NO: 155, CDR-L2 as depicted in SEQ ID NO: 156 and CDR-L3 as depicted in SEQ ID NO: 157,

(m) CDR-H1 as depicted in SEQ ID NO: 167, CDR-H2 as depicted in SEQ ID NO: 168, CDR-H3 as depicted in SEQ ID NO: 169, CDR-L1 as depicted in SEQ ID NO: 170, CDR-L2 as depicted in SEQ ID NO: 171 and CDR-L3 as depicted in SEQ ID NO: 172,

(n) CDR-H1 as depicted in SEQ ID NO: 203, CDR-H2 as depicted in SEQ ID NO: 204, CDR-H3 as depicted in SEQ ID NO: 205, CDR-L1 as depicted in SEQ ID NO: 206, CDR-L2 as depicted in SEQ ID NO: 207 and CDR-L3 as depicted in SEQ ID NO: 208;

(o) CDR-H1 as depicted in SEQ ID NO: 214, CDR-H2 as depicted in SEQ ID NO: 215, CDR-H3 as depicted in SEQ ID NO: 216, CDR-L1 as depicted in SEQ ID NO: 217, CDR-L2 as depicted in SEQ ID NO: 218 and CDR-L3 as depicted in SEQ ID NO: 219;

(p) CDR-H1 as depicted in SEQ ID NO: 226, CDR-H2 as depicted in SEQ ID NO: 227, CDR-H3 as depicted in SEQ ID NO: 228, CDR-L1 as depicted in SEQ ID NO: 229, CDR-L2 as depicted in SEQ ID NO: 230 and CDR-L3 as depicted in SEQ ID NO: 231;

(q) CDR-H1 as depicted in SEQ ID NO: 238, CDR-H2 as depicted in SEQ ID NO: 239, CDR-H3 as depicted in SEQ ID NO: 240, CDR-L1 as depicted in SEQ ID NO: 241, CDR-L2 as depicted in SEQ ID NO: 242 and CDR-L3 as depicted in SEQ ID NO: 243; and

(r) CDR-H1 as depicted in SEQ ID NO: 248, CDR-H2 as depicted in SEQ ID NO: 249, CDR-H3 as depicted in SEQ ID NO: 250, CDR-L1 as depicted in SEQ ID NO: 251, CDR-L2 as depicted in SEQ ID NO: 252 and CDR-L3 as depicted in SEQ ID NO: 253.

12. The pharmaceutical composition of claim 1, wherein the concentration of the bispecific antigen-binding molecule is 10 to 35 or 15 to 31 mg/ml, more preferably 20 to 30 mg/ml or 25 to 30 mg/ml.

13. The pharmaceutical composition of claim 1, wherein the concentration of EDTA is in the range of 0.01% to 0.2% (w/v), preferably in the range of 0.01% to 0.16% (w/v), more preferably 0.04% (w/v).

14. The pharmaceutical composition of claim1, wherein the at least one buffer agent is an acid selected from the group consisting of acetate, glutamate, citrate, succinate, tartrate, fumarate, maleate, histidine, phosphate, 2-(N-morpholino)ethanesulfonate or a combination thereof, preferably glutamate.

15. The pharmaceutical composition of claim 14, wherein the at least one buffer agent is present at a concentration range of 5 to 200 mM, more preferably at a concentration range of 10 to 50 mM, preferably 15 mM.

16. The pharmaceutical composition of claim 1, wherein the at least one saccharide is selected from the group consisting of monosaccharide, disaccharide, cyclic polysaccharide, sugar alcohol, linear branched dextran or linear non-branched dextran.

17. The pharmaceutical composition of claim 16, wherein the disaccharide is selected from the group consisting of sucrose and trehalose and a combination thereof, preferably sucrose.

18. The pharmaceutical composition of claim 16, wherein the sugar alcohol is selected from the group consisting of mannitol and sorbitol and combination thereof.

19. The pharmaceutical composition of claim 16 wherein the at least one saccharide is present at a concentration in the range of 1 to 15% (w/v), preferably in a concentration range of 8 to 12% (w/v), such as 8% (w/v).

20. The pharmaceutical composition of claim 1, further comprising at least one surfactant is selected from the group consisting of polysorbate 20, polysorbate 40,
polysorbate 60, polysorbate 80, poloxamer 188, pluronic F68, triton X-100, polyoxyethylen, PEG 3350, PEG 4000 and combinations thereof.

21. The pharmaceutical composition of claim 1, wherein the composition comprises at least one surfactant at a concentration in the range of 0.004 to 0.5 % (w/V), preferably in the range of 0.01 to 0.1% (w/v).

22. The pharmaceutical composition of claim 1, wherein the pH of the composition is in the range of 4.0 to 5.3, preferably 4.2 to 5.2, more preferably 4.3 to 4.6.

23. The pharmaceutical composition of claim 1, having an osmolarity is in the range of 150 to 500 mOsm.

24. The pharmaceutical composition of claim 1, further comprising an excipient selected from the group consisting of one or more polyol, preferably hydroxypropyl-ß-cyclodextrin, and one or more amino acid, preferably phenylalanine, but preferably not arginine, proline, and tryptophane.

25. The pharmaceutical composition of claim 24, wherein said one or more excipient is present in the concentration range of 0.1 to 15 % (w/v).

26. The pharmaceutical composition of claim 1, wherein the composition comprises
(a) the bispecific antigen-binding molecule of any one of the preceding claims,
(b) 15 mM glutamate or acetate,
(c) 8% (w/V) sucrose or 8% (w/V) sucrose and 1% (w/V) hydroxypropyl-β-cyclodextrin,
(d) optionally 0.01% (w/V) polysorbate 80
and wherein the pH of the liquid pharmaceutical composition is any value in the range of 4.0 to 5.2, preferably 4.2 to 4.6, preferably 4.3 to 4.6.

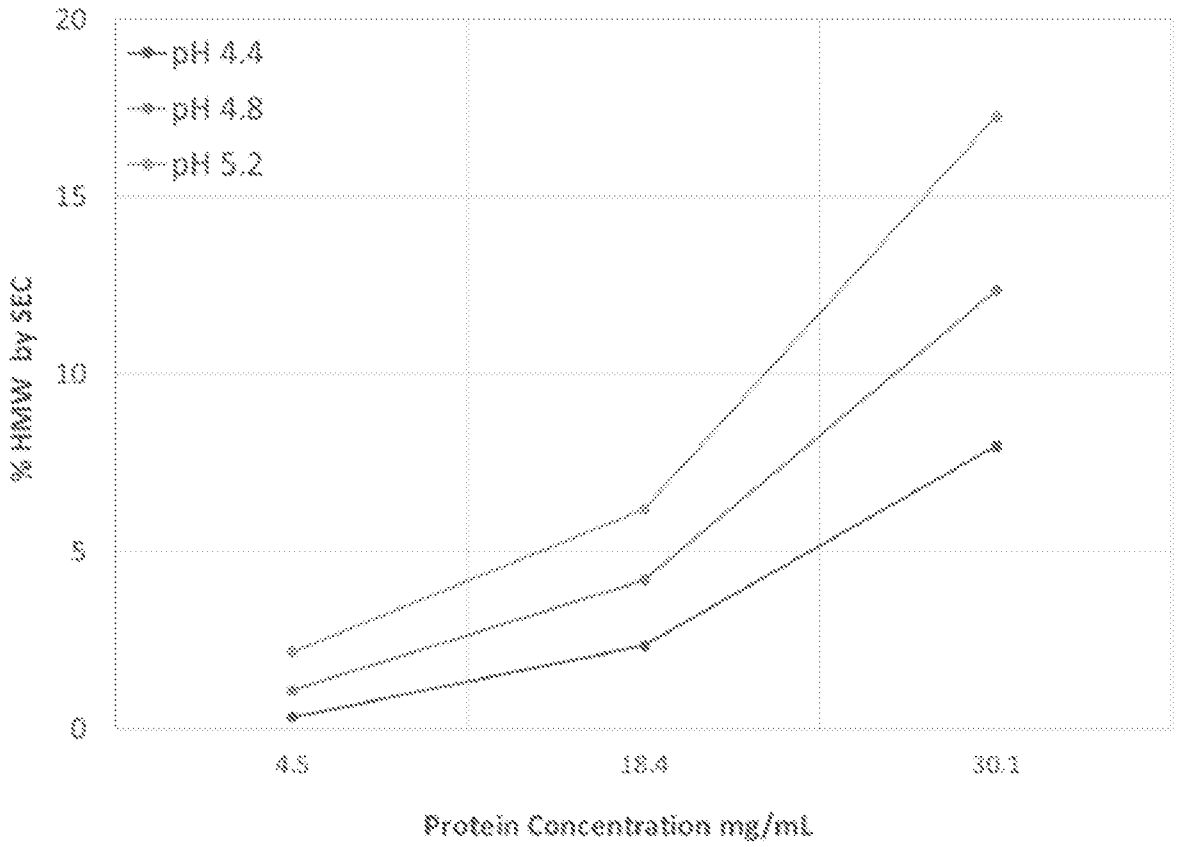
27. A solid pharmaceutical composition, obtainable by lyophilization of the liquid pharmaceutical composition of any one of the preceding claims.

28. A liquid pharmaceutical composition obtainable by reconstituting the solid pharmaceutical composition of claim 27 with a pharmaceutically acceptable liquid.

29. The pharmaceutical composition of any one of the preceding claims for use in the treatment of a disease, preferably of a proliferative disease.

30. Use of the pharmaceutical composition of claim 1 for reducing the formation of high molecular weight species (HMWS) during storage, wherein the amount of HMWS is kept below 5%, preferably below 3% or 2%, if the liquid pharmaceutical composition is stored at or below 4°C, preferably at or below -30°C.

Fig. 1


20
15
10
5
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pH 4.8
pH 5.2
% HMW by SEC
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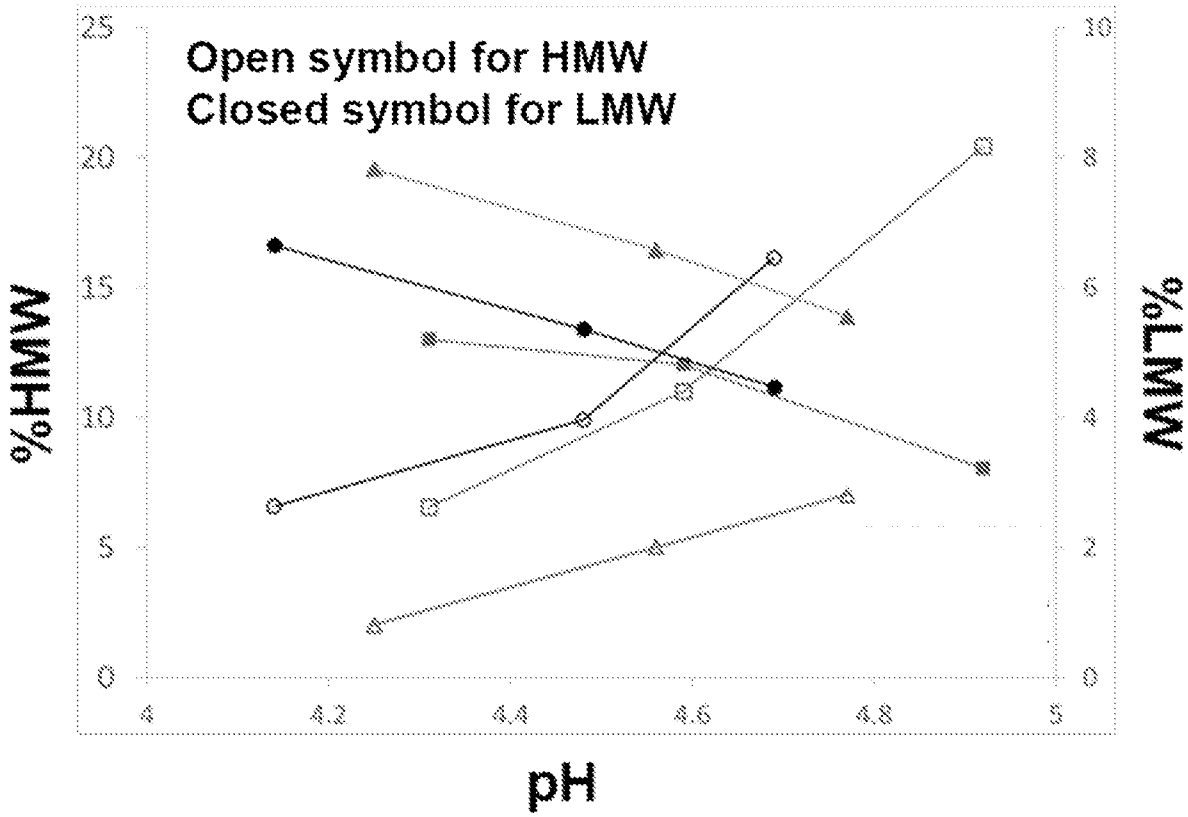
18.4
30.1
Protein Concentration mg/mL

Fig. 2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Closed symbol for LMW
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%LMW
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0
4
4.2
4.4
4.6
4.8
5

Fig. 3

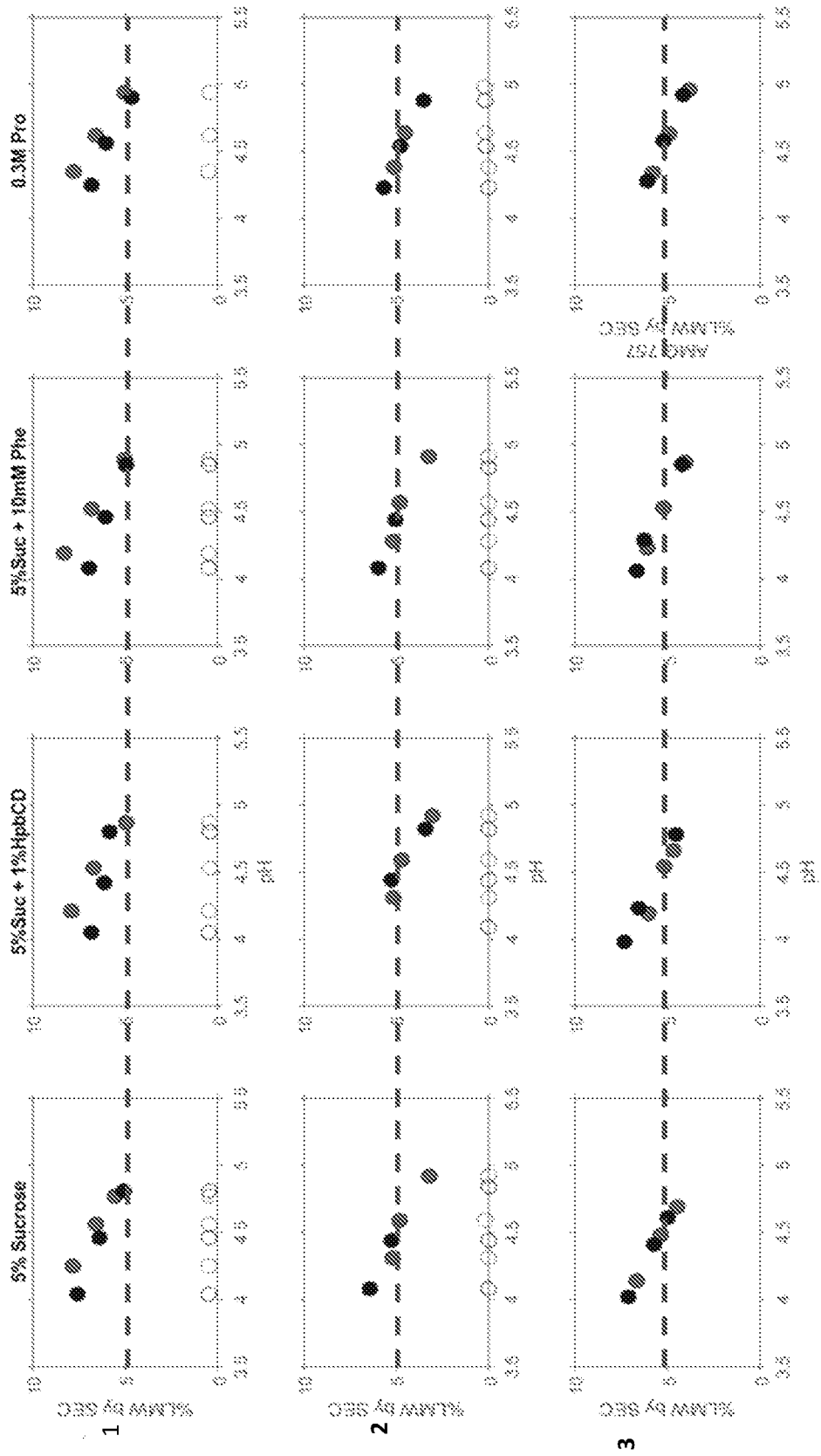
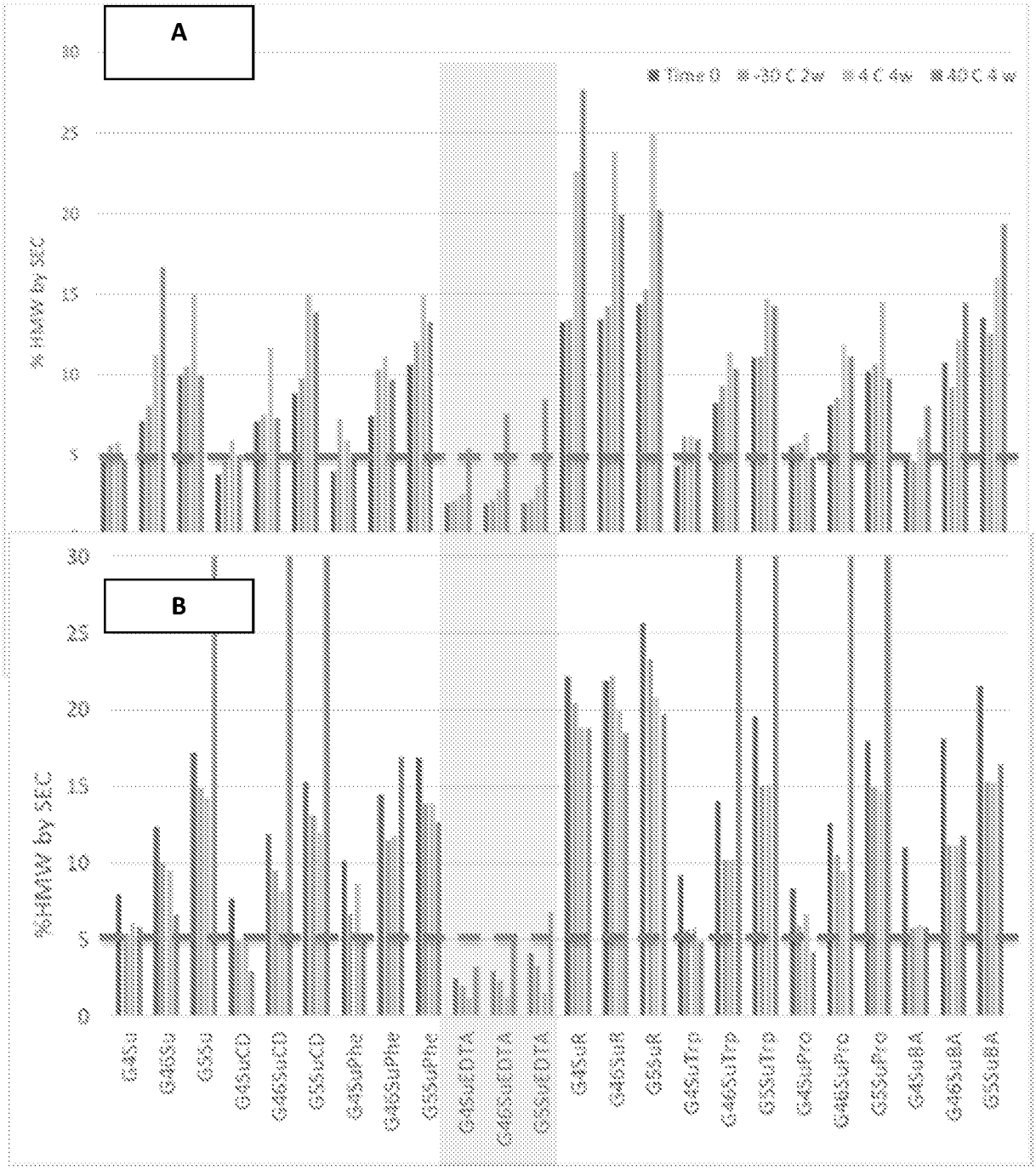


Fig. 4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4°C 4w
40°C 4 w
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5
10
15
20
25
30
B
%HMW by SEC
0
5
10
15
20
25
30
G4Su
G46Su
G5Su
G4SuCD
G46SuCD
G5SuCD
G4SuPhe
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G5SuPhe
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G5SuR
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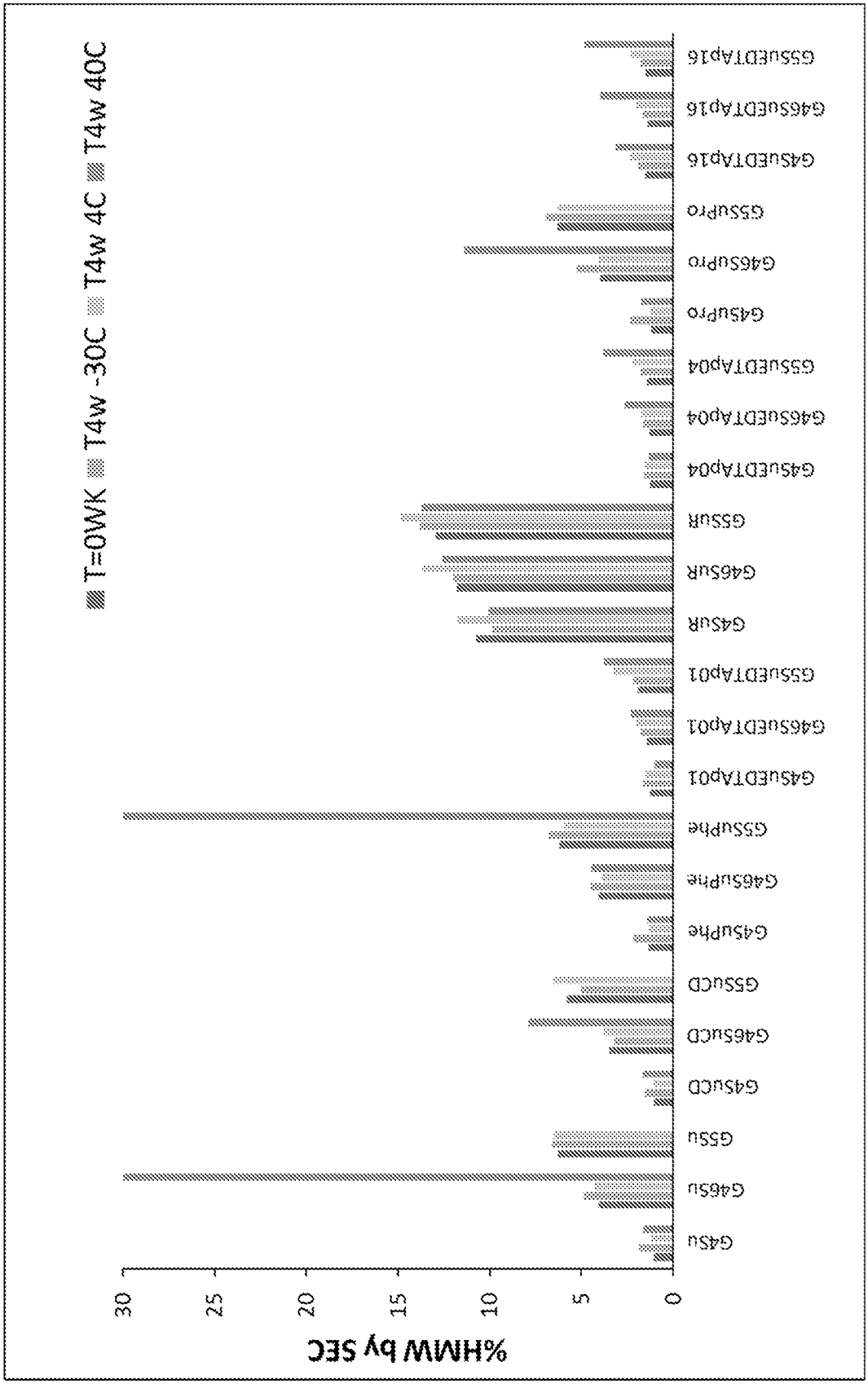
G5SuBA

Fig. 5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20
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G5Su
G4SuCD
G46SuCD
G5SuCD
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