NOVEL PEPTIDES

Field of Invention

The present invention relates to novel bispecific polypeptides, such as antibodies, and their use in the treatment of cancers, particularly cancers expressing carcinoembryonic antigen (CEA).

Background

Immunotherapy of cancer

Cancer is a leading cause of premature deaths in the developed world. Immunotherapy of cancer aims to mount an effective immune response against tumour cells. This may be achieved by, for example, breaking tolerance against tumour antigen, augmenting anti-tumour immune responses, and stimulating local cytokine responses at the tumour site. The key effector cell of a long-lasting anti-tumour immune response is the activated tumour-specific effector T cell. Potent expansion of activated tumour-specific effector T cells can redirect the immune response towards the tumour. In this context, various immunosuppressive mechanisms induced by the tumour microenvironment suppress the activity of effector T cells. Several immunosuppressive mediators are expressed by the tumour cells. Such mediators inhibit T cell activation, either directly, or indirectly by inducing e.g. regulatory T cells (Treg) or myeloid-derived suppressor cells. Depleting, inhibiting, reverting or inactivating such regulatory cells may therefore provide anti-tumour effects and revert the immune suppression in the tumour microenvironment. Further, incomplete activation of effector T cells by, for example, dendritic cells (DC) can result in sub-optimally activated or anergic T cells, resulting in an inefficient anti-tumour response. In contrast, adequate induction by DC can generate a potent expansion of activated effector T cells, redirecting and enhancing the immune response towards the tumour. In addition, natural killer (NK) cells play an important role in tumour immunology by attacking tumour cells with down-regulated human leukocyte antigen (HLA) expression and by inducing antibody dependent cellular cytotoxicity (ADCC). Stimulation of NK cells may thus also reduce tumour growth.

CD40

CD40, a 48 kDa transmembrane cell surface glycoprotein, is a co-stimulatory receptor belonging to the tumor necrosis factor receptor (TNFR) superfamily (Banchereau J, Bazan F, Blanchard D, et al. The CD40 antigen and its ligand. Annu Rev Immunol. 1994;12:881-922; Elgueta R, Benson MJ, de Vries VC, et al. Molecular mechanism and

function of CD40/CD40L engagement in the immune system. Immunol Rev. 2009 May;229(1):152-72). CD40 is expressed in diverse cell types and can be detected on antigen-presenting cells (APC), including dendritic cells (DC), B cells, and macrophages. In addition, CD40 is expressed on granulocytes, endothelial cells, smooth muscle cells, fibroblasts, and epithelial cells (Banchereau J, Bazan F, Blanchard D, et al. The CD40 antigen and its ligand. Annu Rev Immunol. 1994;12:881-922; Elgueta R, Benson MJ, de Vries VC, et al. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev. 2009 May;229(1):152-72; Korniluk A, Kemona H, Dymicka-Piekarska V. Multifunctional CD40L: pro- and anti-neoplastic activity. Tumour Biol. 2014 Oct;35(10):9447-57.; Peters AL, Stunz LL, Bishop GA. CD40 and autoimmunity: the dark side of a great activator. Semin Immunol. 2009 Oct;21(5):293-300.). Consistent with its widespread expression on normal cells, CD40 is also present on the membranes of a wide range of malignant cells, including non-Hodgkin and Hodgkin lymphomas, myelomas, and certain types of carcinomas, including those of the nasopharynx, bladder, cervix, kidney, and ovary (Elgueta R, Benson MJ, de Vries VC, et al. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev. 2009 May;229(1):152-72.; Eliopoulos AG, Young LS. The role of the CD40 pathway in the pathogenesis and treatment of cancer. Curr Opin Pharmacol. 2004 Aug;4(4):360-7).

CD40 interacts with a single ligand, CD40L (CD154), a transmembrane protein that is expressed by activated T cells, but also on B cells, platelets, mast cells, macrophages, basophils, natural killer (NK) cells, and non-hematopoietic cells (smooth muscle cells, endothelial cells, and epithelial cells) (Elgueta R, Benson MJ, de Vries VC, et al. Molecular mechanism and function of CD40/CD40L engagement in the immune system. Immunol Rev. 2009 May;229(1):152-72.; Korniluk A, Kemona H, Dymicka-Piekarska V. Multifunctional CD40L: pro- and anti-neoplastic activity. Tumour Biol. 2014 Oct;35(10):9447-57). The binding of CD40 to CD40L, as part of a cell-cell interaction, activates an intracellular signal transduction pathway that involves a series of adapter molecules known as TNFR activation factors (TRAF). To initiate this intracellular signal transduction, multiple CD40 receptor trimers must form a higher order cluster on the cell membrane (Peters AL, Stunz LL, Bishop GA. CD40 and autoimmunity: the dark side of a great activator. Semin Immunol. 2009 Oct;21(5):293-300; Werneburg BG, Zoog SJ, Dang TT, et al. Molecular characterization of CD40 signaling intermediates. J Biol Chem. 2001 Nov 16;276(46):43334-42). The CD40 clustering forms a signaling complex that allows multiple TRAF to assemble, which in turn leads to the activation of downstream transcription factors, including NFκB (Elgueta R, Benson MJ, de Vries VC, et al. Molecular mechanism and function of CD40/CD40L engagement in the

immune system. Immunol Rev. 2009 May;229(1):152-72; Kornbluth RS, Stempniak M, Stone GW. Design of CD40 agonists and their use in growing B cells for cancer immunotherapy. Int Rev Immunol. 2012 Aug;31(4):279-88).

The molecular consequences of CD40 signaling depend on the cell type expressing CD40 and their microenvironment (Vonderheide RH, Glennie MJ. Agonistic CD40 antibodies and cancer therapy. Clin Cancer Res. 2013 Mar 01;19(5):1035-43). The 'licensing' of APC, in particular DC, results in up-regulation of membrane co-stimulatory molecules and MHC, as well as the production of pro-inflammatory cytokines (Caux C, Massacrier C, Vanbervliet B, et al. Activation of human dendritic cells through CD40 cross-linking. J Exp Med. 1994 Oct 1;180(4):1263-72; van Kooten C, Banchereau J. Functions of CD40 on B cells, dendritic cells and other cells. Curr Opin Immunol. 1997 Jun;9(3):330-7). Thus, CD40 is involved in the functional maturation of APC and consequently the activation of antigen-specific T cells (Ma DY, Clark EA. The role of CD40 and CD154/CD40L in dendritic cells. Semin Immunol. 2009 Oct;21(5):265-72; Moran AE, Kovacsovics-Bankowski M, Weinberg AD. The TNFRs OX40, 4-1BB, and CD40 as targets for cancer immunotherapy. Curr Opin Immunol. 2013 Apr;25(2):230-7). CD40 also plays a role in humoral immunity by activating resting B cells and by increasing their antigen-presenting function (Vonderheide RH, Glennie MJ. Agonistic CD40 antibodies and cancer therapy. Clin Cancer Res. 2013 Mar 01;19(5):1035-43; Zarnegar B, He JQ, Oganesyan G, et al. Unique CD40-mediated biological program in B cell activation requires both type 1 and type 2 NF-kappaB activation pathways. Proc Natl Acad Sci U S A. 2004 May 25;101(21):8108-13). Moreover, CD40 is involved in the induction of innate immunity through stimulation of cells such as macrophages, granulocytes and NK cells (Rakhmilevich AL, Alderson KL, Sondel PM. T-cell-independent antitumor effects of CD40 ligation. Int Rev Immunol. 2012 Aug;31(4):267-78).

Monoclonal CD40 agonist antibodies are believed to trigger anti-tumor effects via two distinct mechanisms: (i) tumor-specific immune activation; and (ii) direct tumoricidal effects via e.g., apoptosis, antibody-dependent cellular cytotoxicity (ADCC), and/or complement-dependent cytotoxicity (CDC) (Khong A, Nelson DJ, Nowak AK, et al. The use of agonistic anti-CD40 therapy in treatments for cancer. Int Rev Immunol. 2012 Aug;31(4):246-66). Treatment with CD40 agonists induces activation of several different immune cells that contribute to the anti-tumor immune response. T cells, and in particular cytotoxic T lymphocytes (CTL), are essential for the anti-tumor effects induced by CD40 agonists, as demonstrated in a range of preclinical models (Byrne KT, Vonderheide RH. CD40 Stimulation Obviates Innate Sensors and Drives T Cell

Immunity in Cancer. Cell Rep. 2016 Jun 21;15(12):2719-32; Mangsbo SM, Broos S, Fletcher E, et al. The human agonistic CD40 antibody ADC-1013 eradicates bladder tumors and generates T-cell-dependent tumor immunity. Clin Cancer Res. 2015 Mar 01;21(5):1115-26; Tutt AL, O'Brien L, Hussain A, et al. T Cell Immunity to Lymphoma Following Treatment with Anti-CD40 Monoclonal Antibody. The Journal of Immunology. 2002;168(6):2720-2728; van Mierlo GJ, den Boer AT, Medema JP, et al. CD40 stimulation leads to effective therapy of CD40(-) tumors through induction of strong systemic cytotoxic T lymphocyte immunity. Proc Natl Acad Sci U S A. 2002 Apr 16;99(8):5561-6). Activation of DC and subsequent priming of T cells likely plays a central role, as the presence of antigen cross-presenting DC is required for the anti-tumor effects of CD40 agonist treatment in T cell-dependent models (Beatty GL, Chiorean EG, Fishman MP, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011 Mar 25;331(6024):1612-6; Beatty GL, Li Y, Long KB. Cancer immunotherapy: activating innate and adaptive immunity through CD40 agonists. Expert Rev Anticancer Ther. 2017 Feb;17(2):175-186; Long KB, Gladney WL, Tooker GM, et al. IFNgamma and CCL2 Cooperate to Redirect Tumor-Infiltrating Monocytes to Degrade Fibrosis and Enhance Chemotherapy Efficacy in Pancreatic Carcinoma. Cancer Discov. 2016 Apr;6(4):400-413; Lum HD, Buhtoiarov IN, Schmidt BE, et al. In vivo CD40 ligation can induce T-cell-independent antitumor effects that involve macrophages. J Leukoc Biol. 2006 Jun;79(6):1181-92). NK cells are also capable of cytotoxic killing of tumor cells, and have been shown to contribute to the reduction in tumor growth in response to a CD40 agonist (Turner JG, Rakhmilevich AL, Burdelya L, et al. Anti-CD40 Antibody Induces Antitumor and Antimetastatic Effects: The Role of NK Cells. The Journal of Immunology. 2001;166(1):89). B cells activated through CD40 can further add to the anti-tumor immune response by presenting antigen to T cells and producing tumor-targeting antibodies (Jackaman C, Cornwall S, Graham PT, et al. CD40-activated B cells contribute to mesothelioma tumor regression. Immunol Cell Biol. 2011 Feb;89(2):255-67; Liu M, Sun Q, Wang J, et al. A New Perspective: Exploring Future Therapeutic Strategies For Cancer By Understanding The Dual Role Of B Lymphocytes In Tumor Immunity. Int J Cancer. 2018 Sep 5). Additionally, CD40 agonists have been found to convert tumor-associated macrophages (TAM) to activated macrophages with anti-tumor properties that can promote tumor shrinkage, independent of T cells (Beatty GL, Chiorean EG, Fishman MP, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011 Mar 25;331(6024):1612-6; Beatty GL, Li Y, Long KB. Cancer immunotherapy: activating innate and adaptive immunity through CD40 agonists. Expert Rev Anticancer Ther. 2017 Feb;17(2):175-186; Long KB, Gladney WL, Tooker GM, et al. IFNgamma and

CCL2 Cooperate to Redirect Tumor-Infiltrating Monocytes to Degrade Fibrosis and Enhance Chemotherapy Efficacy in Pancreatic Carcinoma. Cancer Discov. 2016 Apr;6(4):400-413; Lum HD, Buhtoiarov IN, Schmidt BE, et al. In vivo CD40 ligation can induce T-cell-independent antitumor effects that involve macrophages. J Leukoc Biol. 2006 Jun;79(6):1181-92).

DC are the most important APC for the generation of antigen-specific T cell responses (Flamar AL, Xue Y, Zurawski SM, et al. Targeting concatenated HIV antigens to human CD40 expands a broad repertoire of multifunctional CD4+ and CD8+ T cells. AIDS. 2013 Aug 24;27(13):2041-51). Their central role in inducing anti-tumor immune responses has been shown in preclinical models, where mice deficient in Batf3 and thereby lacking cross-presenting DC (cDC1), show impaired rejection of immunogenic tumors and fail to respond to immunotherapy due to impaired priming of tumor-targeting CTL (Hildner K, Edelson BT, Purtha WE, et al. Batf3 deficiency reveals a critical role for CD8alpha+ dendritic cells in cytotoxic T cell immunity. Science. 2008 Nov 14;322(5904):1097-100; Sanchez-Paulete AR, Cueto FJ, Martinez-Lopez M, et al. Cancer Immunotherapy with Immunomodulatory Anti-CD137 and Anti-PD-1 Monoclonal Antibodies Requires BATF3-Dependent Dendritic Cells. Cancer Discov. 2016 Jan;6(1):71-9). In accordance with these data, the presence of cross-presenting DC in human tumors correlates with CD8+ T cell infiltration and is associated with better prognosis as well as better response to immunotherapy (Broz ML, Binnewies M, Boldajipour B, et al. Dissecting the tumor myeloid compartment reveals rare activating antigen-presenting cells critical for T cell immunity. Cancer Cell. 2014 Nov 10;26(5):638-52; Sanchez-Paulete AR, Teijeira A, Cueto FJ, et al. Antigen Cross-Presentation and T-Cell Cross-Priming In Cancer Immunology And Immunotherapy. Ann Oncol. 2017 Sep 01). Signaling through CD40 on DC induces activation of the antigen presentation machinery and upregulation of co-stimulatory molecules such as CD80 and CD86, thereby improving the capacity of the DC to present antigen to and activate T cells (Beatty GL, Li Y, Long KB. Cancer immunotherapy: activating innate and adaptive immunity through CD40 agonists. Expert Rev Anticancer Ther. 2017 Feb;17(2):175-186; Gladue RP, Paradis T, Cole SH, et al. The CD40 agonist antibody CP-870,893 enhances dendritic cell and B-cell activity and promotes anti-tumor efficacy in SCID-hu mice. Cancer Immunol Immunother. 2011 Jul;60(7):1009-17), and to produce cytokines, notably IL-12, that helps shape the T cell response.

CD40 expression can be detected on all blood DC, with the highest expression found on a subpopulation referred to as cDC1 (Carenza C, Calcaterra F, Oriolo F, et al. Costimulatory Molecules and Immune Checkpoints Are Differentially Expressed on

Different Subsets of Dendritic Cells [Original Research]. Frontiers in Immunology. 2019 2019-June-11;10(1325); MacDonald KP, Munster DJ, Clark GJ, et al. Characterization of human blood dendritic cell subsets. Blood. 2002 Dec 15;100(13):4512-20). Recent studies have focused on the role of cDC1 in driving T cell responses to tumors, demonstrating a potential for CD40 agonists alone or in combination with other therapies in enhancing cDC1 priming of tumor-targeting T cells (Hegde S, Krisnawan VE, Herzog BH, et al. Dendritic Cell Paucity Leads to Dysfunctional Immune Surveillance in Pancreatic Cancer. Cancer Cell. 2020 Mar 16;37(3):289-307 e9; Morrison AH, Diamond MS, Hay CA, et al. Sufficiency of CD40 activation and immune checkpoint blockade for T cell priming and tumor immunity. Proc Natl Acad Sci U S A. 2020 Mar 25; Zhang L, Li Z, Skrzypczynska KM, et al. Single-Cell Analyses Inform Mechanisms of Myeloid-Targeted Therapies in Colon Cancer. Cell. 2020;181(2):442-459.e29). Single-cell RNA sequencing studies confirm the presence of cDC1 with the potential to respond to CD40 agonists in primary tumor tissue (Chevrier S, Levine JH, Zanotelli VRT, et al. An Immune Atlas of Clear Cell Renal Cell Carcinoma. Cell. 2017;169(4):736-749.e18; Zhang L, Li Z, Skrzypczynska KM, et al. Single-Cell Analyses Inform Mechanisms of Myeloid-Targeted Therapies in Colon Cancer. Cell. 2020;181(2):442-459.e29; Zhang Q, He Y, Luo N, et al. Landscape and Dynamics of Single Immune Cells in Hepatocellular Carcinoma. Cell. 2019;179(4):829-845.e20). Targeting CD40 on DC therefore has the capacity to expand the tumor-specific T cell pool, and potentially represents a way to treat immunologically "cold" tumors.

Carcinoembryonic antigen (CEA)

Carcinoembryonic antigen (CEA) describes a family of highly-related glycoproteins (some of which are glycosyl phosphatidyl inositol (GPI) cell-surface-anchored), which are involved in cell functions, such as cell adhesion, phagocytosis, proliferation and signal transduction. CEAs are generally characterised as being members of the CD66 family of molecules (with CEA including examples of CD66a, CD66b, CD66c, CD66d, CD66e, and CD66f molecules). Currently 29 CEA family genes have been identified, which are generally referred to as carcinoembryonic antigen-related cell adhesion molecule (CEACAMs). Examples of the CEACAM genes are *CEACAM1, CEACAM3, CEACAM4, CEACAM5, CEACAM6, CEACAM7, CEACAM8, CEACAM16, CEACAM18, CEACAM19, CEACAM20*, and *CEACAM21*.

CEA (and, in particular, CEACAM5) is usually produced during the development of a fetus, and is only present at very low levels in the blood of a healthy, human, adult. However, in cancer the levels of CEA found are increased, and in that context it is characterised as a tumour-associated antigen (TAA). CEA has been associated with

many types of cancers and tumours, including gastric carcinoma, pancreatic carcinoma, lung carcinoma, breast carcinoma, and medullary thyroid carcinoma. Of particular relevance to cancer and tumours are CEACAM1, CEACAM6, CEACAM7 and CEACAM5 (Zi-Wen Han, Zhi-Wu Lyv, Bin Cui, et al. The old CEACAMs find their new role in tumor immunotherapy. Invest New Drugs volume. 2020 38:1888–1898; Chaogu Zheng, Jing Feng1, Di Lu1, et al. A Novel Anti-CEACAM5 Monoclonal Antibody, CC4, Suppresses Colorectal Tumor Growth and Enhances NK Cells-Mediated Tumor Immunity. PLoS One. 2011;6(6):e21146).

Despite progress in the development of immunotherapies for the treatment of various cancers over the last decade, there remains a need for new and efficacious agents for treating cancers, in particular cancers expressing CEA.

Accordingly, the present invention seeks to provide improved polypeptide-based therapies for the treatment of cancer, in particular cancers expressing CEA.

Summary of the invention

In a first aspect, the present invention provides a bispecific polypeptide comprising a first binding domain, designated B1, which is capable of binding specifically to CD40, and a second binding domain, designated B2, which is capable of specifically binding to CEA.

Such bispecific compounds can be used to establish a highly effective and safe cancer immunotherapy.

Various types of tumour-localizing immunotherapeutic molecules, such as immunocytokines and bispecific antibodies have shown beneficial immune activation and inhibition of tumor growth in preclinical studies as well as in the clinic (reviewed in Kiefer and Neri, 2016).

The clinical progress with immunocytokines has so far not been impressive and the side effects still remain since the tumor-binding entity only confers limited tumor localization, with the bulk of the immunocytokine ending up in other compartments. Bispecific antibodies that restrict the activity to the tumor as described in this invention would provide a clear advantage over immunocytokines since they are inactive in the absence of cancer and/or tumours, in particular cancer and/or tumours that express CEA.

To avoid affecting part of the immune system not relevant for inducing tumour immunity and avoid systemic toxicity by CD40-activating agents, yet obtain high efficacy in the tumour area, the designs of the molecular formats of CD40 agonists may be optimised. For example, a good efficacy/safety profile can be obtained by a CD40-CEA bispecific antibody that requires crosslinking by binding to the CEA for CD40 activation to occur. Thus, CD40-expressing cells such as dendritic cells, residing in the tumour tissue, will preferentially be activated, whereas CD40-expressing cells in other tissues, where the expression of CEA is low or absent, will not. This would allow focused activation of CD40-expressing cells specifically in the tumour tissue, while limiting toxicity induced by generalised CD40 activation.

Structure of the bispecific polypeptide

A "polypeptide" is used herein in its broadest sense to refer to a compound of two or more subunit amino acids, amino acid analogs, or other peptidomimetics. The term "polypeptide" thus includes short peptide sequences and also longer polypeptides and proteins. As used herein, the term "amino acid" refers to either natural and/or unnatural or synthetic amino acids, including both D or L optical isomers, and amino acid analogs and peptidomimetics.

The term "bispecific" as used herein means the polypeptide is capable of specifically binding at least two target entities. Accordingly, bispecific as used herein can describe polypeptides that are capable of specifically binding more than two target entities, such as: at least three, at least four or at least five target entities. In a preferred embodiment, the bispecific polypeptide is capable of specifically binding two target entities.

Thus, the first and/or second binding domains may be selected from the group consisting of antibodies and antigen-binding fragments thereof, and CD40 ligands.

By "an antibody or an antigen-binding fragment thereof" we include substantially intact antibody molecules, as well as chimeric antibodies, humanised antibodies, isolated human antibodies, single chain antibodies, bispecific antibodies, antibody heavy chains, antibody light chains, homodimers and heterodimers of antibody heavy and/or light chains, and antigen-binding fragments and derivatives of the same. Suitable antigen-binding fragments and derivatives include Fv fragments (e.g. single chain Fv and disulphide-bonded Fv), Fab-like fragments (e.g. Fab fragments, Fab' fragments and F(ab)2 fragments), single variable domains (e.g. VH and VL domains) and single

domain antibodies (dAbs, including single and dual formats [i.e. dAb-linker-dAb], and nanobodies). The potential advantages of using antibody fragments, rather than whole antibodies, are several-fold. The smaller size of the fragments may lead to improved pharmacological properties, such as better penetration of solid tissue. Moreover, antigen-binding fragments such as Fab, Fv, ScFv and dAb antibody fragments can be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of the said fragments.

In one preferred embodiment, the polypeptide is a bispecific antibody (numerous examples of which are described in detail below).

In one embodiment, the antigen-binding fragment is selected from the group consisting of: Fv fragments (such as a single chain Fv fragment, or a disulphide-bonded Fv fragment), Fab-like fragments (such as a Fab fragment; a Fab' fragment or a $F(ab)_2$ fragment) and single domain antibodies.

The phrase "an antibody or an antigen-binding fragment thereof" is also intended to encompass antibody mimics (for example, non-antibody scaffold structures that have a high degree of stability yet allow variability to be introduced at certain positions). Those skilled in the art of biochemistry will be familiar with many such molecules, as discussed in Gebauer & Skerra, 2009 (the disclosures of which are incorporated herein by reference). Exemplary antibody mimics include: affibodies (also called Trinectins; Nygren, 2008, *FEBS J*, 275, 2668-2676); CTLDs (also called Tetranectins; *Innovations Pharmac. Technol.* (2006), 27-30); adnectins (also called monobodies; *Meth. Mol. Biol.*, 352 (2007), 95-109); anticalins (*Drug Discovery Today* (2005), 10, 23-33); DARPins (ankyrins; *Nat. Biotechnol.* (2004), 22, 575-582); avimers (*Nat. Biotechnol.* (2005), 23, 1556-1561); microbodies (*FEBS J*, (2007), 274, 86-95); peptide aptamers (*Expert. Opin. Biol. Ther.* (2005), 5, 783-797); Kunitz domains (*J. Pharmacol. Exp. Ther.* (2006) 318, 803-809); affilins (*Trends. Biotechnol.* (2005), 23, 514-522); affimers (Avacta Life Sciences, Wetherby, UK).

Also included within the scope of the invention are chimeric T cell receptors (also known as chimeric immunoreceptors, and chimeric antigen receptors or CARs) (see Pule *et al.*, 2003, the disclosures of which are incorporated herein by reference). These are engineered receptors, which graft an arbitrary specificity onto an immune effector cell. Typically, CARs are used to graft the specificity of a monoclonal antibody onto a T cell; with transfer of their coding sequence facilitated by retroviral vectors. The most common form of such molecules is fusions comprising a single-chain variable fragment

(scFv) derived from a monoclonal antibody fused to CD3-zeta transmembrane and endodomain. When T cells express this fusion molecule, they recognize and kill target cells that express the transferred monoclonal antibody specificity.

Persons skilled in the art will further appreciate that the invention also encompasses modified versions of antibodies and antigen-binding fragments thereof, whether existing now or in the future, *e.g.* modified by the covalent attachment of polyethylene glycol or another suitable polymer (see below).

Methods of generating antibodies and antibody fragments are well known in the art. For example, antibodies may be generated via any one of several methods which employ induction of *in vivo* production of antibody molecules, screening of immunoglobulin libraries (Orlandi. *et al*, 1989; Winter *et al*., 1991, the disclosures of which are incorporated herein by reference) or generation of monoclonal antibody molecules by cell lines in culture. These include, but are not limited to, the hybridoma technique, the human B cell hybridoma technique, and the Epstein-Barr virus (EBV)-hybridoma technique (Kohler *et al*., 1975, Kozbor *et al*., 1985; Cote *et al*., 1983; Cole *et al*., 1984., the disclosures of which are incorporated herein by reference).

Suitable methods for the production of monoclonal antibodies are also disclosed in *"Monoclonal Antibodies: A manual of techniques"*, H Zola (CRC Press, 1988, the disclosures of which are incorporated herein by reference) and in *"Monoclonal Hybridoma Antibodies: Techniques and Applications"*, J G R Hurrell (CRC Press, 1982, the disclosures of which are incorporated herein by reference).

Likewise, antibody fragments can be obtained using methods well known in the art (see, for example, Harlow & Lane, 1988, *"Antibodies: A Laboratory Manual"*, Cold Spring Harbor Laboratory, New York, the disclosures of which are incorporated herein by reference). For example, antibody fragments according to the present invention can be prepared by proteolytic hydrolysis of the antibody or by expression in *E. coli* or mammalian cells (*e.g*. Chinese hamster ovary cell culture or other protein expression systems) of DNA encoding the fragment. Alternatively, antibody fragments can be obtained by pepsin or papain digestion of whole antibodies by conventional methods.

It will be appreciated by persons skilled in the art that for human therapy or diagnostics, human or humanised antibodies are preferably used. Humanised forms of non-human (*e.g.* murine) antibodies are genetically engineered chimeric antibodies or antibody fragments having preferably minimal-portions derived from non-human

antibodies. Humanised antibodies include antibodies in which complementary determining regions of a human antibody (recipient antibody) are replaced by residues from a complementary determining region of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired functionality. In some instances, Fv framework residues of the human antibody are replaced by corresponding non-human residues. Humanised antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported complementarity determining region or framework sequences. In general, the humanised antibody will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the complementarity determining regions correspond to those of a non-human antibody and all, or substantially all, of the framework regions correspond to those of a relevant human consensus sequence. Humanised antibodies optimally also include at least a portion of an antibody constant region, such as an Fc region, typically derived from a human antibody (see, for example, Jones *et al.*, 1986, Riechmann *et al.*, 1988, Presta, 1992, the disclosures of which are incorporated herein by reference).

Methods for humanising non-human antibodies are well known in the art. Generally, the humanised antibody has one or more amino acid residues introduced into it from a source which is non-human. These non-human amino acid residues, often referred to as imported residues, are typically taken from an imported variable domain. Humanisation can be essentially performed as described (see, for example, Jones *et al.*, 1986, Reichmann *et al.*, 1988, Verhoeyen *et al.*, 1988, US 4,816,567, the disclosures of which are incorporated herein by reference) by substituting human complementarity determining regions with corresponding rodent complementarity determining regions. Accordingly, such humanised antibodies are chimeric antibodies, wherein substantially less than an intact human variable domain has been substituted by the corresponding sequence from a non-human species. In practice, humanised antibodies may be typically human antibodies in which some complementarity determining region residues and possibly some framework residues are substituted by residues from analogous sites in rodent antibodies.

Human antibodies can also be identified using various techniques known in the art, including phage display libraries (see, for example, Hoogenboom & Winter, 1991, Marks *et al.*, 1991, Cole *et al.*, 1985, Boerner *et al.*, 1991, the disclosures of which are incorporated herein by reference).

It will be appreciated by persons skilled in the art that the bispecific polypeptides, *e.g.* antibodies, of the present invention may be of any suitable structural format.

Thus, in exemplary embodiments of the bispecific antibodies of the invention:

(a) binding domain B1 and/or binding domain B2 is an intact IgG antibody (or, together, form an intact IgG antibody);

(b) binding domain B1 and/or binding domain B2 is an Fv fragment (*e.g.* an scFv);

(c) binding domain B1 and/or binding domain B2 is a Fab fragment; and/or

(d) binding domain B1 and/or binding domain B2 is a single domain antibody (*e.g.* domain antibodies and nanobodies).

It will be appreciated by persons skilled in the art that the bispecific antibody may comprise a human Fc region, or a variant of a said region, where the region is an IgG1, IgG2, IgG3 or IgG4 region, preferably an IgG1 or IgG4 region.

Engineering the Fc region of a therapeutic monoclonal antibody or Fc fusion protein allows the generation of molecules that are better suited to the pharmacology activity required of them (Strohl, 2009, the disclosures of which are incorporated herein by reference).

By "CD40 ligands", we include non-antibody molecules that are capable of binding to CD40; for example CD40L (CD154, such as GenBank: D31797.2) or fragments or variants of CD40L that retain their ability to bind to CD40.

(a) *Engineered Fc regions for increased half-life*

One approach to improve the efficacy of a therapeutic antibody is to increase its serum persistence, thereby allowing higher circulating levels, less frequent administration and reduced doses.

The half-life of an IgG depends on its pH-dependent binding to the neonatal receptor FcRn. FcRn, which is expressed on the surface of endothelial cells, binds the IgG in a pH-dependent manner and protects it from degradation.

Some antibodies that selectively bind the FcRn at pH 6.0, but not pH 7.4, exhibit a higher (to put another way longer) half-life in a variety of animal models. Additionally, some antibodies that bind the FcRn with a higher affinity at pH 6.0, but with a remained low affinity at pH 7.4 exhibit a longer half-life.

Several mutations located at the interface between the CH2 and CH3 domains, such as T250Q/M428L (Hinton *et al.*, 2004, the disclosures of which are incorporated herein by reference) and M252Y/S254T/T256E + H433K/N434F (Vaccaro et *al.*, 2005, the disclosures of which are incorporated herein by reference), have been shown to increase the binding affinity to FcRn and the half-life of IgG1 *in vivo*.

(b) *Engineered Fc regions for altered effector function*

To ensure lack of CD40 activation in the absence of CEA, the Fc portion of the bispecific antibody should bind with no or very low affinity to FcγR, since FcγR-mediated crosslinking of a CD40 antibody may induce activation. By "very low affinity" we include that the Fc portion exhibits at least 10 times reduced affinity to FcγRI, FcγRII and III compared to wild-type IgG1, as determined by the concentration where half maximal binding is achieved in flow cytometric analysis of FcγR expressing cells (Hezareh *et al.*, 2001) or by FcγR ELISA (Shields *et al.*, 2001).

Another factor to take into account is that engagement of FcγRs may also induce antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and complement-dependent cytotoxicity (CDC) of cells coated with antibodies. In one embodiment, to enhance tumour-dependent CD40 activation as well as to avoid depletion of CD40-expressing cells, the isotype of a CD40-CEA bispecific antibody should preferably be silent.

The four human IgG isotypes bind the activating Fcγ receptors (FcγRI, FcγRIIa, FcγRIIIa), the inhibitory FcγRIIb receptor, and the first component of complement (C1q) with different affinities, yielding very different effector functions (Bruhns *et al.*, 2009, the disclosures of which are incorporated herein by reference). IgG1 molecules have the highest affinity and capacity to induce effector functions, whereas IgG2, IgG3 and IgG4 are less effective (Bruhns, 2012; Hogarth and Pietersz, 2012; Stewart *et al.*, 2014) (Wang *et al.* 2015; Vidarson *et al.* 2014). In addition, certain mutations in the Fc region of IgG1 dramatically reduce FcγR affinity and effector function while retaining neonatal FcR (FcRn) interaction (Ju and Jung, 2014; Leabman *et al.*, 2013; Oganesyan *et al.,* 2008; Sazinsky *et al.,* 2008).

The most widely used IgG1 mutants are N297A alone or in combination with D265A, as well as mutations at positions L234 and L235, including the so-called "LALA" double mutant L234A/L235A. Another position described to further silence IgG1 by mutation is P329 (see US 2012/0251531).

Thus, choosing a mutated IgG1 format with low effector function but retained binding to FcRn may result in a bispecific antibody with CEA-dependent activation of CD40, and exhibiting a favorable efficacy/safety profile and good PK properties.

Advantageously, the polypeptide is incapable of inducing antibody-dependent cell cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), and/or complement-dependent cytotoxicity (CDC). By "incapable" we include that the ability of the polypeptide to induce ADCC, *etc.*, is at least 10-fold lower than compared to wild-type IgG1 as shown by e.g. monocyte-dependent ADCC or CDC assays described by Hezareh *et al*. 2001.

In one embodiment, the Fc region may be a variant of a human IgG1 Fc region comprising a mutation at one or more of the following positions:

L234, L235, P239, D265, N297 and/or P329.

Advantageously, alanine may be present at the mutated position(s).

Optionally, the IgG1 variant may be a variant of a human IgG1 Fc region comprising mutations L234A and L235A (i.e. the LALA double mutant; see SEQ ID NO: 336).

It will be appreciated by persons skilled in the art that the bispecific polypeptides of the invention may be of several different structural formats (for example, see Chan & Carter, 2016, the disclosures of which are incorporated herein by reference).

In exemplary embodiments, the bispecific antibody is selected from the groups consisting of:

(a) bivalent bispecific antibodies, such as IgG-scFv bispecific antibodies (for example, wherein B1 is an intact IgG and B2 is an scFv attached to B1 at the N-terminus of a light chain and/or at the C-terminus of a light chain and/or at the N-terminus of a heavy chain and/or at the C-terminus of a heavy chain of the IgG, or *vice versa*);

(b) monovalent bispecific antibodies, such as a *DuoBody*® (Genmab AS, Copenhagen, Denmark) or 'knob-in-hole' bispecific antibody (for example, an scFv-KIH, scFv-KIH^r, a BiTE-KIH or a BiTE-KIH^r (see Xu *et al*., 2015, *mAbs* 7(1):231-242));

(c) $scFv_2$-Fc bispecific antibodies (such as *ADAPTIR*™ bispecific antibodies from Aptevo Therapeutics);

(d) $BiTE/scFv_2$ bispecific antibodies;

(e) DVD-Ig bispecific antibodies;

(f) DART-based bispecific antibodies (for example, $DART_2$-Fc or DART);

(g) $DNL\text{-}Fab_3$ bispecific antibodies; and

(h) scFv-HSA-scFv bispecific antibodies.

For example, the bispecific antibody may be an IgG-scFv antibody. The IgG-scFv antibody may be in either VH-VL or VL-VH orientation. In one embodiment, the scFv may be stabilised by a S-S bridge between VH and VL.

In one embodiment, binding domain B1 and binding domain B2 are fused directly to each other.

In an alternative embodiment, binding domain B1 and binding domain B2 are joined via a polypeptide linker. For example, a polypeptide linker may be a short linker peptide between about 10 to about 25 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*.

Thus, the linker may be selected from the group consisting of the amino acid sequence SGGGGSGGGGS (SEQ ID NO: 337), SGGGGSGGGGSAP (SEQ ID NO: 338), NFSQP (SEQ ID NO: 339), KRTVA (SEQ ID NO: 340), GGGSGGGG (SEQ ID NO: 341), GGGGSGGGGS, (SEQ ID NO: 342), GGGGSGGGGSGGGGS (SEQ ID NO: 343), GSTSGSGKPGSGEGSTKG (SEQ ID NO: 344) (Whitlow *et al.* 1993) THTCPPCPEPKSSDK (SEQ ID NO: 345), GGGS (SEQ ID NO: 346), EAAKEAAKGGGGS (SEQ ID NO: 347), EAAKEAAK (SEQ ID NO: 348), or (SG)m, where m = 1 to 7.

In a preferred embodiment, the linker may be selected from the group consisting of: SEQ ID NO: 341, SEQ ID NO: 342 and SEQ ID NO: 343. In a particularly preferred embodiment, the linker is GGGGSGGGGSGGGGS (SEQ ID NO: 343).

The term "amino acid" as used herein includes the standard twenty genetically-encoded amino acids and their corresponding stereoisomers in the 'D' form (as compared to the natural 'L' form), omega-amino acids other naturally-occurring amino acids, unconventional amino acids (*e.g.* α,α-disubstituted amino acids, N-alkyl amino acids, *etc.*) and chemically derivatised amino acids (see below).

When an amino acid is being specifically enumerated, such as "alanine" or "Ala" or "A", the term refers to both L-alanine and D-alanine unless explicitly stated otherwise. Other unconventional amino acids may also be suitable components for polypeptides of the present invention, as long as the desired functional property is retained by the polypeptide. For the peptides shown, each encoded amino acid residue, where appropriate, is represented by a single letter designation, corresponding to the trivial name of the conventional amino acid.

In one embodiment, the polypeptides as defined herein comprise or consist of L-amino acids.

It will be appreciated by persons skilled in the art that the polypeptides of the invention may comprise or consist of one or more amino acids which have been modified or derivatised.

Chemical derivatives of one or more amino acids may be achieved by reaction with a functional side group. Such derivatised molecules include, for example, those molecules in which free amino groups have been derivatised to form amine hydrochlorides, *p*-toluene sulphonyl groups, carboxybenzoxy groups, *t*-butyloxycarbonyl groups, chloroacetyl groups or formyl groups. Free carboxyl groups may be derivatised to form salts, methyl and ethyl esters or other types of esters and hydrazides. Free hydroxyl groups may be derivatised to form O-acyl or O-alkyl derivatives. Also included as chemical derivatives are those peptides which contain naturally occurring amino acid derivatives of the twenty standard amino acids. For example: 4-hydroxyproline may be substituted for proline; 5-hydroxylysine may be substituted for lysine; 3-methylhistidine may be substituted for histidine; homoserine may be substituted for serine and ornithine for lysine. Derivatives also include peptides containing one or more additions or deletions as long as the requisite activity is maintained. Other included modifications are amidation, amino terminal acylation (*e.g.* acetylation or thioglycolic acid amidation), terminal carboxylamidation (*e.g.* with ammonia or methylamine), and the like terminal modifications.

It will be further appreciated by persons skilled in the art that peptidomimetic compounds may also be useful. The term 'peptidomimetic' refers to a compound that mimics the conformation and desirable features of a particular peptide as a therapeutic agent.

For example, the said polypeptide includes not only molecules in which amino acid residues are joined by peptide (-CO-NH-) linkages but also molecules in which the peptide bond is reversed. Such retro-inverso peptidomimetics may be made using methods known in the art, for example such as those described in Meziere *et al.* (1997), which is incorporated herein by reference. This approach involves making pseudo-peptides containing changes involving the backbone, and not the orientation of side chains. Retro-inverse peptides, which contain NH-CO bonds instead of CO-NH peptide bonds, are much more resistant to proteolysis. Alternatively, the said polypeptide may be a peptidomimetic compound wherein one or more of the amino acid residues are linked by a -y(CH_2NH)- bond in place of the conventional amide linkage.

In a further alternative, the peptide bond may be dispensed with altogether provided that an appropriate linker moiety which retains the spacing between the carbon atoms of the amino acid residues is used; it may be advantageous for the linker moiety to have substantially the same charge distribution and substantially the same planarity as a peptide bond.

It will also be appreciated that the said polypeptide may conveniently be blocked at its N- or C-terminus so as to help reduce susceptibility to exo-proteolytic digestion.

A variety of un-coded or modified amino acids such as D-amino acids and N-methyl amino acids have also been used to modify mammalian peptides. In addition, a presumed bioactive conformation may be stabilised by a covalent modification, such as cyclisation or by incorporation of lactam or other types of bridges, for example see Veber *et al.*, 1978 and Thursell *et al.*, 1983, which are incorporated herein by reference.

In one embodiment of the invention, one of binding domain B1 or binding domain B2 is an immunoglobulin molecule, and one of binding domain B1 or binding domain B2 is a Fab fragment, wherein the Fab fragment is fused to the C terminus of the heavy chain of the immunoglobulin via the light chain of the Fab fragment.

For example, the polypeptide may have a format as shown in Figure 23. Such a format is referred to as the "RUBY™ format" (as described in pending UK patent application 1820556.7 and the PCT application WO 2020/127354). Antibodies in the "RUBY™ format" and "optimised RUBY™ format", as described herein, are particularly preferred, for the bispecific polypeptides of the invention.

The bispecific polypeptide may comprise one or more mutations to promote association of the heavy chain polypeptide of the immunoglobulin with the light chain polypeptide of the immunoglobulin and/or to promote association of the heavy chain polypeptide of the Fab with the light chain polypeptide of the Fab.

In one embodiment the one or more mutations prevent the formation of aggregates and a Fab by-product.

It will be appreciated by persons skilled in the art, that in one embodiment the mutations may prevent the formation of aggregates and/or a Fab by-product by generating steric hindrance and/or incompatibility between charges.

By "steric hindrance" we mean the slowing of a reaction due to steric bulk, i.e. the size of an amino acid molecule prevents association of two protein surfaces that may otherwise occur if a smaller amino acid is present.

By "incompatibility between charges" we mean that an unwanted product will not form as the charges are incompatible and prevent the product from forming, e.g. there may be two negatively charged portions which repel and prevent an unwanted product from forming.

As described above, said mutations limit the formation of a Fab by-product and/or aggregates by, for example, creating surfaces that limit the formation of aggregates or by-product Fab fragments. In one embodiment, the mutations prevent formation of a Fab by-product by generating steric hindrance and/or incompatibility between charges (leading to charge incompatibility of wrong chains). The mutations may also promote interactions between correct chains (i.e. between the first heavy chain polypeptide and the first light chain polypeptide, and/or between the second heavy chain polypeptide and the second light chain polypeptide) by, for example, creating salt or disulphide bridges.

Thus, the mutations may favour formation of the bispecific polypeptide.

In one embodiment, the percentage of aggregates formed during manufacturing is less than or equal to 25%. Optionally the percentage of aggregates is less than or equal to 20%, 17.5%, 15%, 13.5% or 10%. Preferably the percentage of aggregates is less than 10%. Optionally these measurements are carried out when the chains of the

bispecific polypeptide are transfected at equal ratios, e.g. at a ratio of 1:1:1 when 3 chains are used during production.

Alternatively, the chain transfection ratio may be optimised. Optionally the % of aggregates when the chain transfection ratio is optimised may be less than or equal to 3.5%, 3%, 2.5% or 2%.

In one embodiment, the bispecific polypeptide comprises one or more mutation pairs each comprising two functionally compatible mutations.

By "functionally compatible mutations" we mean the mutations have complementary functions, e.g. one mutation of the pair (in one chain) may be a mutation that forms a positively charged region, and the other mutation (in another chain) forms a negatively charged region. Together these mutations act in a functionally compatible way promoting association of the respective chains.

In one embodiment, the bispecific polypeptide comprises one or more mutation pairs in one or more of the following region groups:

(a) the CH1 and CKappa or CLambda region of the immunoglobulin; and/or
(b) the CH1 and CKappa or CLambda region of the Fab; and/or
(c) the VL and VH regions of the immunoglobulin; and/or
(d) the VL and VH regions of the Fab.

Thus, in one embodiment, the mutation pairs are in the CH1 and CKappa or CLambda regions of the Fab and/or the immunoglobulin, and the mutation pairs are selected from:

(a) cavity and protruding surface mutations (i.e. steric mutations); and/or
(b) hydrophobic swap mutations; and/or
(c) charged mutations (i.e. salt mutations); and/or
(d) mutations resulting in the formation of a disulphide bridge.

The mutation pairs may alternatively or additionally be in the VH and VL regions of the Fab and/or the immunoglobulin, the mutation pairs in the VH and VL regions are selected from:

(a) charged mutations (i.e. salt mutations); and/or
(b) double charged mutations; and/or
(c) mutations resulting in the formation of a disulphide bridge.

In one embodiment of the invention the mutations are at positions selected from the group consisting of:

(a) one or more of the following positions in the CH1 domain: H168, F170, L145, S183 and T187 (according to EU numbering system); and/or
(b) a position selected from the one or more of the following position ranges in the CKappa or CLambda domain: position 132 to 138, position 173 to 179, position 130 to 136, position 111 to 117 and position 134 to 140 (according to EU numbering system); and/or
(c) a position selected from one or more of the following position ranges in the VL: position 41 to 47, position 117 to 123 and position 46 to 52 (according to IMGT numbering system); and/or
(d) a position selected from one or more of the following position ranges in the VH: position 41 to 47, position 46 to 52 and position 117 to 123 (according to IMGT numbering system).

In one embodiment of the invention the mutations are at positions selected from the group consisting of:

(a) one or more of the following positions in the CH1 domain: H168, F170, L145, S183 and T187 (according to EU numbering system); and/or
(b) a position selected from the one or more of the following position ranges in the CKappa or CLambda domain: position 132 to 138, position 173 to 179, position 130 to 136, position 111 to 117 and position 134 to 140 (according to Kabat numbering system); and/or
(c) a position selected from one or more of the following position ranges in the VL: position 41 to 47, position 117 to 123 and position 46 to 52 (according to IMGT numbering system); and/or
(d) a position selected from one or more of the following position ranges in the VH: position 41 to 47, position 46 to 52 and position 117 to 123 (according to IMGT numbering system).

In one embodiment of the invention the mutations are at positions selected from the group consisting of:

(a) one or more of the following positions in the CH1 domain: H168, F170, L145, S183 and T187 (according to EU numbering system); and/or

(b) a position selected from the one or more of the following position ranges in the CKappa or CLambda domain: position 132 to 138, position 173 to 179, position 130 to 136, position 111 to 117 and position 134 to 140 (according to EU numbering system); and/or

(c) a position selected from one or more of the following position ranges in the VL: position 41 to 47, position 117 to 123 and position 46 to 52 (according to IMGT numbering system); and/or

(d) a position selected from one or more of the following position ranges in the VH: position 41 to 47, position 46 to 52 and position 117 to 123 (according to IMGT numbering system).

One mutation in each of the ranges given above will be the relevant functional mutation as it will be a position that makes contact with the amino acid in the corresponding domain/chain, and is therefore the relevant interface between chains.

It will therefore be appreciated by persons skilled in the art that mutations in the position ranges given above are suitable, as the relevant functional feature is whether the position contacts a corresponding position on the other chain, i.e. a position in the VH chain that contacts a corresponding position in a VL chain is the relevant position, or a position in a CLambda that contacts a position in a CH1 chain is the relevant position.

In one embodiment the mutations are selected from the group consisting of:

VH X44R/E/D/K, X49C, X120K

VL X44R/E/D/K, X49D X120C

CH1 H168A/G, F170G/A, L145Q, S183V, T187E/D,

CKappa/ CLambda S/T114A, V133T, L135Y/W, N/S137K/R/H, S176W/V/Y

*numbering according to IMGT system for VH/VL domains and according to EU numbering system for constant domains

*X refers to any amino acid

The use of "/" in the context of discussing mutations is to illustrate alternative possible amino acids; for example, "X44R/E/D/K" indicates that R or E or D or K can be included at position 44, as a substitute for the amino acid "X".

In one embodiment the mutations are selected from the group consisting of:

VH X44R/E/D/K, X49C, X120K

VL X44R/E/D/K, X49D X120C

CH1 H168A/G, F170G/A, L145Q, S183V, T187E/D,
CKappa/ CLambda S/T114A, V133T, L135Y/W, N/S137K/R/H, S176W/V/Y
*numbering according to IMGT system for VH/VL domains and according to Kabat numbering system for constant domains
*X refers to any amino acid

In one embodiment of the invention, the mutations are at positions selected from the group consisting of:

(a) one or more of the following positions in the CH1 domain: H168, F170, L145, S183 and T187 (according to EU numbering system); and/or
(b) one or more of the following positions in the CKappa domain: L135, S176, V133, S114 and N137 (according to EU numbering system) and/or one or more of the following positions in the CLambda domain: L135, S176, V133, T114 and S137 (according to EU numbering system); and/or
(c) one or more of the following positions in the VL: Q44, Q120 and A49 (according to IMGT numbering system); and/or
(d) one or more of the following positions in the VH: Q44, G49 and Q120 (according to IMGT numbering system).

In one embodiment of the invention, the mutations are at positions selected from the group consisting of:

(a) one or more of the following positions in the CH1 domain: H168, F170, L145, S183 and T187 (according to EU numbering system); and/or
(b) one or more of the following positions in the CKappa domain: L135, S176, V133, S114 and N137 (according to Kabat numbering system) and/or one or more of the following positions in the CLambda domain: L135, S176, V133, T114 and S137 (according to Kabat numbering system); and/or
(c) one or more of the following positions in the VL: Q44, Q120 and A49 (according to IMGT numbering system); and/or
(d) one or more of the following positions in the VH: Q44, G49 and Q120 (according to IMGT numbering system).

For example, the mutations may be selected from the group consisting of:

(a) one or more of the following mutations in the CH1 domain: H168A, F170G, L145Q, S183V and T187E (according to EU numbering system); and/or

(b) one or more of the following mutations in the CKappa domain: L135Y, S176W, V133T, S176V, S114A and N137K (according to EU numbering system) and/or one or more of the following mutations in the CLambda domain: L135Y, S176W, V133T, S176V, T114A and S137K (according to EU numbering system); and/or

(c) one or more of the following mutations in the VL: Q44R, Q44E, Q120C, Q44D and A49D (according to IMGT numbering system); and/or

(d) one or more of the following mutations in the VH: Q44E, Q44R, G49C, Q44K and Q120K (according to IMGT numbering system).

For example, the mutations may be selected from the group consisting of:

(a) one or more of the following mutations in the CH1 domain: H168A, F170G, L145Q, S183V and T187E (according to EU numbering system); and/or

(b) one or more of the following mutations in the CKappa domain: L135Y, S176W, V133T, S176V, S114A and N137K (according to Kabat numbering system) and/or one or more of the following mutations in the CLambda domain: L135Y, S176W, V133T, S176V, T114A and S137K (according to Kabat numbering system); and/or

(c) one or more of the following mutations in the VL: Q44R, Q44E, Q120C, Q44D and A49D (according to IMGT numbering system); and/or

(d) one or more of the following mutations in the VH: Q44E, Q44R, G49C, Q44K and Q120K (according to IMGT numbering system).

The above mutations are those of the "RUBY™ format".

In a further embodiment, the polypeptide may have a format as shown in Figure 23 with further optimised mutations, which is referred to as the "optimised RUBY™ format".

Although bispecific polypeptides in the "RUBY™ format" can be reproducibly produced with an excellent level of purity, bispecific polypeptides in the "optimised RUBY™ format" can be reproducibly produced at an even higher level of purity. Further, bispecific polypeptides in the "optimised RUBY™ format" have been engineered to carry a reduced risk of provoking immunogenic responses directed against the bispecific polypeptide itself.

The optimised mutations are described below as "optimised mutation set 1" and "optimised mutation set 2" – including "set 2a" and/or "set 2b". It will be appreciated by the skilled person various combinations of these optimised mutations could be used

in a bispecific polypeptide of the invention, as well as in combination with any of the "RUBY™ format" mutations described above. The combinations of the "RUBY™ format" mutations and "optimised RUBY™ format" mutations, used in the same bispecific antibody, are described below. It will also be appreciated that the variations of those mutations as described herein would also work as part of the invention. All mutations in variable domains (VH or VL) are numbered according to the IMGT numbering system, and all mutations in the constant domains are numbered according to the EU numbering system.

Mutation set 1 - Mutations in the variable domain heavy (VH):
T65E, T65A, T65I.

Mutation set 2 - any individual and/or any combination of the mutations listed in set 2a and set 2b. Set 2a - mutations in the CH1: Y180A, Y180G, Y180I, Y180N, Y180S, Y180T, Y180V, or Y180W, and/or S183N or S183T, and/or V188G; preferably, Y180T. Set 2b - mutations in the CKappa domain: A111R, A111T, A111W or A111V, and/or T109P; preferably: T109P and/or A111V; and/or mutations in the variable domain light (VL): I126A, I126G, I126H, I126N, I126P, I126Q, I126S, or I126T.

In one embodiment of the invention the mutations are at positions selected from the group consisting of:

(a) the T65 position in the VH (according to the IMGT numbering system); and/or
(b) one or more of the following positions in the CH1: Y180; S183; and V188, preferably Y180 (according to the EU numbering system); and/or
(c) one or more of the following positions in the CKappa domain: A111 and T109 (according to the EU or Kabat numbering systems); and/or
(d) the I126 position in the VL (according to the IMGT numbering system).

In a particular embodiment, the mutation is at the T65 position in the variable domain heavy (VH)(according to the IMGT numbering system).

In a particular embodiment, the mutations are one or more of the following positions in the CH1: Y180; S183; and V188, preferably Y180 (according to the EU numbering system).

In a particular embodiment, the mutations are one or more of the following positions in the CKappa domain: A111 and T109 (according to the EU numbering system); and/or the I126 position in the VL (according to the IMGT numbering system).

In one embodiment of the invention the mutations are selected from the group consisting of:

(a) X65E/A/I in the VH (according to the IMGT numbering system); and/or
(b) one or more of the following mutations in the CH1: X180A/G/I/N/S/T/V/W; X183N/T; and X188G; preferably, X180T (according to the EU numbering system); and/or
(c) one or more of the following mutations in the CKappa domain: X111R/T/W/V; and X109P, preferably X111V and X109P (according to the EU or Kabat numbering systems); and/or
(d) X126A/G/H/N/P/Q/S/T in the VL (according to the IMGT numbering system).
*X refers to any amino acid

In a particular embodiment, the mutation is X65E/A/I in the VH (according to the IMGT numbering system).
*X refers to any amino acid

In a particular embodiment, the mutation is one or more of the following mutations in the CH1: X180A/G/I/N/S/T/V/W; X183N/T; and X188G; preferably, X180T (according to the EU numbering system).
*X refers to any amino acid

In a particular embodiment, the mutation is one or more of the following mutations in the CKappa domain: X111R/T/W/V; and X109P, preferably X111V and X109P (according to the EU or Kabat numbering systems); and/or the mutation is X126A/G/H/N/P/Q/S/T in the VL (according to the IMGT numbering system).
*X refers to any amino acid

For example, the mutations may be selected from the group consisting of:

(a) one or more of the following mutations in the VH: T65E; T65A; and T65I (according to the IMGT numbering system); and/or

(b) one or more of the following mutations in the CH1: Y180A; Y180G; Y180I; Y180N; Y180S; Y180T; Y180V; Y180W; S183N; S183T; V188G, preferably Y180T (according to the EU numbering system); and/or

(c) one or more of the following mutations in the CKappa domain: A111R; A111T; A111W; A111V; and T109P, preferably T109P and A111V (according to the EU or Kabat numbering systems); and/or

(d) one or more of the following mutations in the VL: I126A; I126G; I126H; I126N; I126P; I126Q; I126S; and I126T (according to the IMGT numbering system).

In a particular example, the mutations are one or more of the following mutations in the VH: T65E; T65A; and T65I (according to the IMGT numbering system).

In a particular example, the mutations are one or more of the following mutations in the CH1: Y180A; Y180G; Y180I; Y180N; Y180S; Y180T; Y180V; Y180W; S183N; S183T; V188G, preferably Y180T (according to the EU numbering system).

In a particular example, the mutations are one or more of the following mutations in the CKappa domain: A111R; A111T; A111W; A111V; and T109P, preferably T109P and A111V (according to the EU or Kabat numbering systems); and/or one or more of the following mutations in the VL: I126A; I126G; I126H; I126N; I126P; I126Q; I126S; and I126T (according to the IMGT numbering system).

As discussed above, any combination of the "RUBY™ format" mutations and "optimised RUBY™ format" mutations can be used in the same bispecific polypeptide, such as any one or more of the following "RUBY™ format" mutations in (a) to (d), or variations described herein, being combined with any one or more of the following "optimised RUBY™ format" mutations in (e) to (g), or variations described herein:

(a) one or more of the following mutations in the CH1 domain: H168A, F170G and/or T187E (according to EU numbering system);

(b) one or more of the following mutations in the CKappa domain: L135Y, S176W, S114A and/or N137K (according to EU or Kabat numbering systems) and/or one or more of the following mutations in the CLambda domain: L135Y, S176W, T114A and/or S137K (according to Kabat numbering system);

(c) mutations in the VL: Q44R or Q44E (according to IMGT numbering system); and

(d) mutations in the VH: Q44E or Q44R (according to IMGT numbering system);

(e) mutations in the VH: T65E, T65A or T65I (according to IMGT numbering system);

(f) mutation in the CH1: Y180T (according to EU numbering system); and/or

(g) mutations in the CKappa: T109P and/or A111V (according to EU or Kabat numbering systems).

Accordingly, in a particular embodiment, a bispecific antibody with combined "RUBY™ format" mutations and "optimised RUBY™ format" mutations could include the following mutations:

- one or more of the following mutations in the CH1 domain: H168A, F170G, Y180T and/or T187E (according to EU numbering system);
- one or more of the following mutations in the CKappa domain: T109P, A111V, L135Y, S176W, S114A and/or N137K (according to EU or Kabat numbering systems) and/or one or more of the following mutations in the CLambda domain: L135Y, S176W, T114A and/or S137K (according to Kabat numbering system);
- mutations in the VL: Q44R or Q44E (according to IMGT numbering system); and/or
- one or more of the following mutations in the VH: Q44E or Q44R, and/or T65E, T65A or T65I (according to IMGT numbering system).

In one embodiment, the one or more Fab fragment(s) is linked to the C-terminal end of the immunoglobulin via a linker.

In one embodiment of the first aspect of the invention, the bispecific polypeptide is tetravalent, capable of binding bivalently to each of the two antigens.

In one embodiment, the bispecific polypeptide comprises an immunoglobulin arranged as an antibody with two arms and therefore two binding sites for the first antigen, and two of the Fab fragments, each providing a binding site for the second antigen. Thus, there are two binding sites for the first antigen and two binding sites for the second antigen. The bispecific polypeptide of this embodiment may comprise three polypeptide chains: (1) chain H1 which comprises the heavy chain of the IgG a linker and the light chain of a Fab; (2) chain L1 is the light chain for the IgG; and (3) chain H2 is the heavy chain for the appended (attached) Fab. In a preferred embodiment, the bispecific polypeptide may comprise six polypeptide chains: (a) two chain H1, which comprise the heavy chain of the IgG a linker and the light chain of a Fab; (b) two chain L1, which are the light chain for the IgG; and (c) two chain H2, which are the heavy chain for the appended (attached) Fab. This structure can be used for both the "RUBY™ format" and "optimised RUBY™ format" antibodies.

In one embodiment, binding domain B1 is an immunoglobulin and binding domain B2 is a Fab. In an alternative embodiment, binding domain B1 is a Fab and binding domain B2 is an immunoglobulin.

In one embodiment, the bispecific polypeptide may modulate the activity of and/or activate a target immune system cell, wherein said modulation is an increase or decrease in the activity of said cell. Such cells include T cells, dendritic cells and natural killer cells.

In another embodiment, the bispecific polypeptide may modulate the activity of and/or activate myeloid cells, such as macrophages, monocytes and myeloid-derived suppressor cells.

Monocytes and macrophages also express CD40 and may promote immune responses against tumors. Indeed, the murine anti-CD40 surrogate antibody FGK45 was shown to be capable of mediating anti-tumor activity involving macrophages, independent of T cell and NK cell function (Lum HD, Buhtoiarov IN, Schmidt BE, et al. In vivo CD40 ligation can induce T-cell-independent antitumor effects that involve macrophages. J Leukoc Biol. 2006 Jun;79(6):1181-92). However, the effects of CD40 agonists on macrophages and other myeloid cell populations also result in increased production of IFN-γ and CCL5, which promote improved influx of T cells to the tumor (Huffman AP, Lin JH, Kim SI, et al. CCL5 mediates CD40-driven CD4+ T cell tumor infiltration and immunity. JCI Insight. 2020 May 21;5(10)).

Several studies have indicated that CD40 agonist antibodies can convert TAM into activated macrophages with an anti-tumor phenotype. FGK45 interacts with TAM following treatment *in vivo*, and results in their increased expression of MHCII and CD86 (Beatty GL, Chiorean EG, Fishman MP, et al. CD40 agonists alter tumor stroma and show efficacy against pancreatic carcinoma in mice and humans. Science. 2011 Mar 25;331(6024):1612-6). Similar effects have been observed on $CD11b^+$ $F4/80^+$ macrophages in the spleen (Luheshi NM, Coates-Ulrichsen J, Harper J, et al. Transformation of the tumour microenvironment by a CD40 agonist antibody correlates with improved responses to PD-L1 blockade in a mouse orthotopic pancreatic tumour model. Oncotarget. 2016 Apr 5;7(14):18508-20), and the liver, where the treatment may result in hepatotoxicity due to the strong effect on macrophages (Byrne KT, Vonderheide RH. CD40 Stimulation Obviates Innate Sensors and Drives T Cell Immunity in Cancer. Cell Rep. 2016 Jun 21;15(12):2719-32; Medina-Echeverz J, Ma C, Duffy AG, et al. Systemic Agonistic Anti-CD40 Treatment of Tumor-Bearing Mice

Modulates Hepatic Myeloid-Suppressive Cells and Causes Immune-Mediated Liver Damage. Cancer Immunol Res. 2015 May;3(5):557-66). Interestingly, aged and obese mice were shown to be more susceptible to systemic toxicity after immunotherapy such as anti-CD40, and it was further demonstrated that macrophages were the cells primarily responsible for these effects (Bouchlaka MN, Sckisel GD, Chen M, et al. Aging predisposes to acute inflammatory induced pathology after tumor immunotherapy. J Exp Med. 2013 Oct 21;210(11):2223-37; Mirsoian A, Bouchlaka MN, Sckisel GD, et al. Adiposity induces lethal cytokine storm after systemic administration of stimulatory immunotherapy regimens in aged mice. J Exp Med. 2014 Nov 17;211(12):2373-83). Macrophage-mediated hepatotoxicity following anti-CD40 treatment was later shown to be alleviated by combination treatment with anti-CSF-1R antibody, which blocked CSF-1R signalling supporting differentiation, proliferation and function of monocytes and macrophages (Byrne KT, Vonderheide RH. CD40 Stimulation Obviates Innate Sensors and Drives T Cell Immunity in Cancer. Cell Rep. 2016 Jun 21;15(12):2719-32). Combination therapy with anti-CD40 and anti-CSF-1R is currently being explored in clinical studies (Machiels JP, Gomez-Roca C, Michot JM, et al. Phase Ib study of anti-CSF-1R antibody emactuzumab in combination with CD40 agonist selicrelumab in advanced solid tumor patients. J Immunother Cancer. 2020 Oct;8(2)).

The immune system cell (for example, the target immune cell) is typically a dendritic cell. For example, the bispecific polypeptide may be capable of inducing activation of dendritic cells, which are then capable of internalising tumour associated debris or extracellular vesicles containing CEA and tumour neoantigens.

For example, the polypeptide may be capable of inducing:

(a) tumour-specific immune activation; and/or

(b) activation of dendritic cells; and/or

(c) internalisation of associated tumour debris and/or extracellular vesicles containing CEA as well as tumour neoantigens; and/or

(d) cross-presentation of peptides derived from internalised tumour antigens on MHC; and/or

(e) priming and activation of effector T cells; and/or

(f) direct tumoricidal effects, selected from the list consisting of: apoptosis, necroptosis, antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

It will be appreciated by persons skilled in the art, that said activation of dendritic cells may be an increase in the expression of the co-stimulatory molecules CD40, CD80 or CD86, or increased IL-12 production. Alternatively, activation of dendritic cells can be determined by the increased ability to cross-present antigens, e.g. tumour neoantigens, on MHC class I or II to T cells, generating an enhanced activation of T cells recognizing said antigen, by the antigen-presenting cell.

In one embodiment, the bispecific antibody induces an increase in the uptake of tumour debris or tumour extracellular vesicles by an antigen-presenting cell, such as a dendritic cell. It will be appreciated by persons skilled in the art, that said increase in uptake may be measured by the co-localization or internalization of the tumour debris or tumour extracellular vesicles by the antigen-presenting cell.

The increased uptake of tumour debris or tumour extracellular vesicles by the antigen-presenting cells would subsequently result in an effective presentation of neoantigens contained within the tumour debris or tumour extracellular vesicles in the context of MHC molecules, which in turn results in a broader tumor specific T cell repertoire and, thus, more effective T cell-mediated tumour eradication. Methods for determining the expansion of tumour-antigen specific T cells are well known and include, for example, the use of MHC-peptide multimers, e.g. tetramers or pentamers. Such expansion may be measured by inoculating mice with tumours expressing a specific tumour antigen or tumours transfected with a tumour model antigen (*e.g.*, ovalbumin), alternatively by inoculating mice with the same cells that have been heat shocked to induce necrosis, followed by measuring the expansion of tumour antigen-specific T cells by use of various MHC-tumour (model) antigen peptide tetramers or pentamers by flow cytometry-based methods. Alternatively, such expansion may be measured by culturing dendritic cells with antigen-specific TCR transgenic T cells labelled with a proliferative dye and tumour debris or tumour-derived extracellular vesicles derived from tumours transfected with a model antigen (*e.g.*, ovalbumin). Expansion of the antigen-specific T cells can be assessed by analysing dilution of the proliferative dye using flow cytometry.

The polypeptide or binding domains of the invention can also be characterised and defined by their binding abilities. Standard assays to evaluate the binding ability of ligands towards targets are well known in the art, including for example, ELISA, Western blot, RIA, and flow cytometry analysis. The binding kinetics (*e.g.,* binding affinity) of the polypeptide can also be assessed by standard assays known in the art, such as by surface plasmon resonance analysis or bio-layer interferometry.

The terms "binding activity" and "binding affinity" are intended to refer to the tendency of a polypeptide molecule to bind or not to bind to a target. Binding affinity may be quantified by determining the dissociation constant (K_D) for a polypeptide and its target. A lower K_D is indicative of a higher affinity for a target. Similarly, the specificity of binding of a polypeptide to its target may be defined in terms of the comparative dissociation constants (K_D) of the polypeptide for its target as compared to the dissociation constant with respect to the polypeptide and another, non-target molecule.

The value of this dissociation constant can be determined directly by well-known methods, and can be computed even for complex mixtures by methods such as those, for example, set forth in Caceci *et al.*, 1984 (the disclosures of which are incorporated herein by reference). For example, the K_D may be established using a double-filter nitrocellulose filter binding assay such as that disclosed by Wong & Lohman, 1993. Other standard assays to evaluate the binding ability of ligands such as antibodies towards targets are known in the art, including for example, ELISA, Western blot, RIA, and flow cytometry analysis. The binding kinetics (*e.g.,* binding affinity) of the polypeptide also can be assessed by standard assays known in the art, such as by surface plasmon resonance (by use of *e.g.*, Biacore™ system analysis) or by bio-layer interferometry (by use of e.g. Octet® system analysis).

A competitive binding assay can be conducted in which the binding of the polypeptide to the target is compared to the binding of the target by another, known ligand of that target, such as another polypeptide. The concentration at which 50% inhibition occurs is known as the Ki. Under ideal conditions, the Ki is equivalent to K_D. The Ki value will never be less than the K_D, so measurement of Ki can conveniently be substituted to provide an upper limit for K_D.

Alternative measures of binding affinity include EC50 or IC50. In this context EC50 indicates the concentration at which a polypeptide achieves 50% of its maximum binding to a fixed quantity of target. IC50 indicates the concentration at which a polypeptide inhibits 50% of the maximum binding of a fixed quantity of competitor to a fixed quantity of target. In both cases, a lower level of EC50 or IC50 indicates a higher affinity for a target. The EC50 and IC50 values of a ligand for its target can both be determined by well-known methods, for example ELISA. Suitable assays to assess the EC50 and IC50 of polypeptides are set out in the Examples.

A polypeptide of the invention is preferably capable of binding to its target with an affinity that is at least two-fold, 10-fold, 50-fold, 100-fold or greater than its affinity for binding to another non-target molecule.

In one embodiment, the bispecific polypeptide is capable of:

(a) activation of a B-cell, in the presence of a CEA (preferably CEACAM5); and/or
(b) activation of dendritic cells in the presence of CEA (preferably CEACAM5); and/or
(c) capable of increased dendritic cell cross-presentation of neoantigens; and/or
(d) inducing proliferation of neoantigen specific T cells.

In one embodiment, the bispecific polypeptide promotes uptake of tumor derived material, derived from tumor cells overexpressing CEA (preferably CEACAM5). In a particular embodiment, the uptake of tumor derived material is by antigen presenting cells.

It will be appreciated by persons skilled in the art, that said activation of B-cell activation can be characterised by CD86 upregulation, as well as, optionally, other markers of B-cell activation.

CD40 binding domains

The bispecific polypeptides of the invention comprise a binding domain (B1) which is capable of specifically binding to CD40. Preferably, B1 is an agonistic CD40 binding domain.

Binding domain B1 specifically binds to CD40, *i.e.* it binds to CD40 but does not bind, or binds at a lower affinity, to other molecules. The term CD40, as used herein, typically refers to human CD40. The sequence of human CD40 is set out in GenBank: X60592.1. Binding domain B1 may have some binding affinity for CD40 from other mammals, such as CD40 from a non-human primate (for example *Macaca fascicularis* (cynomolgus monkey), *Macaca mulatta*). Binding domain B1 preferably does not bind to murine CD40 and/or does not bind to other human TNFR superfamily members, for example human CD137 or OX40.

Advantageously, binding domain B1 binds to human CD40 with a K_D of less than $2x10^{-7}$M or less than $1.5x10^{-7}$M or less than $8.5x10^{-8}$M or less than $8x10^{-8}$M or less than

$7.5x10^{-8}$M or less than $7x10^{-8}$M or less than $9x10^{-8}$M or less than $9x10^{-9}$M or less than $5x10^{-10}$M or less than $3x10^{-10}$M, preferably less than $8.5x10^{-8}$M, more preferably less than $5x10^{-10}$M or less than $3x10^{-10}$M. Preferably, the K_D is measured in Octet; for example, as explained in the Examples.

For example, binding domain B1 preferably does not bind to murine CD40 or any other TNFR superfamily member, such as CD137 or OX40. Therefore, typically, the K_D for the binding domain with respect to human CD40 will be 2-fold, preferably 5-fold, more preferably 10-fold less than K_D with respect to the other, non-target molecules, such as murine CD40, other TNFR superfamily members, or any other unrelated material or accompanying material in the environment. More preferably, the K_D will be 50-fold less, even more preferably 100-fold less, and yet more preferably 200-fold less.

Binding domain B1 is preferably capable of binding to its target with an affinity that is at least two-fold, 10-fold, 50-fold, 100-fold or greater than its affinity for binding to another non-target molecule.

In summary therefore, binding domain B1 preferably exhibits at least one of the following functional characteristics:

a) binding to human CD40 with a K_D value which is less than $2x10^{-7}$M, more preferably less than $5x10^{-10}$M;

b) does not bind to murine CD40;

c) does not bind to other human TNFR superfamily members, for example human CD137 or OX40.

In one embodiment, binding domain B1 comprises one or more light chain CDR sequences selected from those in Table C(2), and/or one or more heavy chain CDR sequences selected from Table C(1). Thus binding domain B1 may comprise one or more CDR sequences selected from the groups consisting of:

(a) CD40 heavy chain CDRs, SEQ ID NOs: 73 to 89; and/or

(b) CD40 light chain CDRs, SEQ ID NOs: 90 to 104.

In one embodiment binding domain B1 comprises one, two or three light chain CDR sequences from a particular row for an individual antibody reference in Table C(2), and/or one, two or three heavy chain CDR sequences from the corresponding row for the antibody with the same reference in Table C(1). For example, binding domain B1 might comprise one or more of the light chain CDR sequences for 1132 (SEQ ID NOs:

90, 91 and 92) and one or more of the heavy chain CDR sequences for 1132 (SEQ ID NOs: 73, 74 and 75), or binding domain B1 might comprise one or more of the light chain CDR sequences for 1132 (SEQ ID NOs: 96, 97 and 98) and one or more of the heavy chain CDR sequences for 1132 (SEQ ID NOs: 81, 82 and 83). Most preferably, B1 comprises the CDRs and/or the VL and VH of 1132. Also most preferably, B1 comprises the CDRs and/or the VL and VH of G12 or G12-mut.

The CDRs of G12-mut are shared by ffAC_05337. Accordingly, in a preferred embodiment B1 comprises the CDRs of ffAC_05337, which are SEQ ID NOs: 81-83 and 96-98.

Preferred CD40 binding domains may comprise at least a heavy chain CDR3 as defined in any individual row of Table C(1) and/or a light chain CDR3 as defined in in any individual row of Table C(2).

Accordingly, in one embodiment binding domain B1 comprises all six CDR sequences for a given antibody (VH/VL) reference, for example binding domain B1 might comprise all six CDR sequences of antibody 1132 or all six CDR sequences of antibody G12 (as also present in G12_mut and ffAC_05337).

In one embodiment, binding domain B1 comprises a VH and/or a VL amino acid sequence as given in Table A. In one embodiment, binding domain B1 comprises a VH and VL amino acid sequence as given in Table A for a particular antibody reference. For example, binding domain B1 may comprise the VH sequence of 1132 (SEQ ID NO: 3) and/or the VL sequence of 1132 (SEQ ID NO: 1), or the VH sequence of G12 (SEQ ID NO: 19) and/or the VL sequence of G12 (SEQ ID NO: 17), the VH sequence of G12-mut (SEQ ID NO: 29) and/or the VL sequence of G12_mut (SEQ ID NO: 17), the VH sequence of ffAC_05337 (SEQ ID NO: 431) and/or the VL sequence of ffAC_05337 (SEQ ID NO: 430).

In a preferred embodiment B1 comprises the VL and VH of ffAC_05337, which are SEQ ID NO: 430 and 431.

In one embodiment the CD40 binding domain of B1 is selected from: 1132; 1150, 1140, 1107, G12, APX005 and 21.4.1. Preferably, the CD40 binding domain of B1 is G12 and/or 1132. Most preferably, the CD40 binding domain of B1 is G12. In an alternative most preferred embodiment, the CD40 binding domain of B1 is G12_mut.

Thus, the CDR or VH and VL sequences of binding domain B1 might be selected from antibodies from the group consisting of:

(a) 1132 (heavy chain CDRs: SEQ ID NOs: 73, 74 and 75; light chain CDRs: SEQ ID NOs: 90, 91, and 92; VL: SEQ ID NO: 1; VH: SEQ ID NO: 3)

(b) 1150 (heavy chain CDRs: SEQ ID NOs: 73, 76 and 77; light chain CDRs: SEQ ID NOs: 90, 91, and 93; VL: SEQ ID NO: 5; VH: SEQ ID NO: 7)

(c) 1140 (heavy chain CDRs: SEQ ID NOs: 73, 78 and 79; light chain CDRs: SEQ ID NOs: 90, 91, and 94; VL: SEQ ID NO: 9; VH: SEQ ID NO: 11)

(d) 1107 (heavy chain CDRs: SEQ ID NOs: 73, 78 and 80; light chain CDRs: SEQ ID NOs: 90, 91, and 95; VL: SEQ ID NO: 13; VH: SEQ ID NO: 15)

(e) G12 (heavy chain CDRs: SEQ ID NOs: 81, 82 and 83; light chain CDRs: SEQ ID NOs: 96, 97, and 98; VL: SEQ ID NO: 17; VH: SEQ ID NO: 19)

(f) APX005 (heavy chain CDRs: SEQ ID NOs: 84, 85 and 86; light chain CDRs: SEQ ID NOs: 99, 100, and 101; VL: SEQ ID NO: 21; VH: SEQ ID NO: 23)

(g) 21.4.1 (heavy chain CDRs: SEQ ID NOs: 87, 88 and 89; light chain CDRs: SEQ ID NOs: 102, 103, and 104; VL: SEQ ID NO: 25, VH: SEQ ID NO: 27)

(h) G12_mut (heavy chain CDRs: SEQ ID NOs: 81, 82 and 83; light chain CDRs: SEQ ID NOs: 96, 97, and 98; VL: SEQ ID NO: 17; VH: SEQ ID NO: 29)

(i) ffAC_05337 (heavy chain CDRs: SEQ ID NOs: 81, 82 and 83; light chain CDRs: SEQ ID NOs: 96, 97, and 98; VL: SEQ ID NO: 431; VH: SEQ ID NO: 430).

The numbering of the antibody (e.g. Antibody X/Y) defines the heavy chain variable region (X) and the light chain variable region (Y), respectively (or, where a single number is indicated, the heavy chain variable region [X] only is defined). As described above, the sequences may be one or more CDR sequence, or the VH and/or VL sequence. As described above, the sequences of the bispecific polypeptide may comprise specified mutations.

In one embodiment binding domain B1 is specific for CD40, typically human CD40 and may comprise any one, two, three, four, five or all six features independently selected from the following:

(a) a heavy chain CDR1 sequence which consists of the sequence "G, F, T, F, S, S, Y, A";

(b) a heavy chain CDR2 sequence which is 8 amino acids in length and comprises the consensus sequence: "I, G/S, S/G, Y/S, G/S, G/S, G/Y/S, T";

(c) a heavy chain CDR3 sequence which is 9 to 12 amino acids in length and which comprises the consensus sequence of : "A, R, Y/R/G, Y/P/V/-, N/S/V, F/Y/W, G/H/S, -/S, -/V, M/F, D, Y"

(d) a light chain CDR1 sequence which consists of the sequence: "Q, S, I, S, S, Y";

(e) a light chain CDR2 sequence which consists of the sequence: "A, A, S";

(f) a light chain CDR3 sequence which is 9 amino acids in length and comprises the consensus sequence: "Q,Q, Y/S, G/Y, R/S/V, N/A/Y/T, P, P/F/Y, T".

The use of "," in the context of discussing amino acid sequences is to illustrate a list of amino acids when further nomenclature, such as "/", is included; for example, "G, F, T, F, S, S, Y, A" indicates that the sequence of amino acids is GFTFSSYA and "A, R, Y/R/G" indicates that the sequence of amino acids could be ARY or ARR or ARG. The use of "-" in the context of discussing amino acid sequences is to illustrate that there might not be an amino acid present at that respective position; for example, "-/V, M/F, D" indicates that the sequence of amino acids could be VMD or VFD or MD or FD.

Binding domain B1 may comprise at least a heavy chain CDR3 as defined in (c) and/or a light chain CDR3 as defined in (f). Binding domain B1 may comprise all three heavy chain CDR sequences of (a), (b) and (c) and/or all three light chain CDR sequences of (d), (e) and (f).

Examples of complete heavy and light chain variable region amino acid sequences for binding domain B1 are shown in Table A. Exemplary nucleic acid sequences encoding each amino acid sequence are also shown. The numbering of said VH and VL regions in Table A corresponds to the numbering system used as in Table C(1) and C(2). Thus, for example, the amino acid sequence for "1132, light chain VL (also known as 1133)" is an example of a complete VL region sequence comprising all three CDRs of VL number 1132 (1133) shown in Table C(2) and the amino acid sequence for "1132, heavy chain VH" is an example of a complete VH region sequence comprising all three CDRs of VH number 1132 shown in Table C(1).

In exemplary embodiments, binding domain B1 comprises:

(a) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1132/1133 (SEQ ID NOs: 73, 74 and 75; and/or SEQ ID NOs: 90, 91, and 92);

(b) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1150/1151 (SEQ ID NOs: 73, 76 and 77; and/or SEQ ID NOs:90, 91, and 93);

(c) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1140/1135 (SEQ ID NOs: 73, 78 and 79; and/or SEQ ID NOs: 90, 91, and 94);

(d) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1107/1108 (SEQ ID NOs: 73, 78 and 80; and/or SEQ ID NOs: 90, 91, and 95);

(e) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody G12 or G12_mut or ffAC_05337 (SEQ ID NOs: 81, 82 and 83; and/or SEQ ID NOs: 96, 97, and 98);

(f) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody APX005 (SEQ ID NOs: 84, 85 and 86; and/or SEQ ID NOs: 99, 100, and 101); or

(g) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 21.4.1 (SEQ ID NOs: 87, 88 and 89; and/or SEQ ID NOs: 102, 103, and 104).

Thus, binding domain B1 may comprise:

(a) the heavy chain variable region and/or the light chain variable region of antibody 1132/1133 (SEQ ID NO: 3 and/or SEQ ID NO: 1);

(b) the heavy chain variable region and/or the light chain variable region of antibody 1150/1151 (SEQ ID NO: 7 and/or SEQ ID NO: 5);

(c) the heavy chain variable region and/or the light chain variable region of antibody 1140/1135 (SEQ ID NO: 11 and/or SEQ ID NO: 9);

(d) the heavy chain variable region and/or the light chain variable region of antibody 1107/1108 (SEQ ID NO:15 and/or SEQ ID NO: 13);

(e) the heavy chain variable region and/or the light chain variable region of antibody G12 (SEQ ID NO: 19 and/or SEQ ID NO: 17);

(f) the heavy chain variable region and/or the light chain variable region of antibody APX005 (SEQ ID NO: 23 and/or SEQ ID NO: 21);

(g) the heavy chain variable region and/or the light chain variable region of antibody 21.4.1 (SEQ ID NO: 27 and/or SEQ ID NO: 25);

(h) the heavy chain variable region and/or the light chain variable region of antibody G12_mut (SEQ ID NO: 29 and/or SEQ ID NO: 17); or

(h) the heavy chain variable region and/or the light chain variable region of antibody ffAC_05337 (SEQ ID NO: 431 and/or SEQ ID NO: 430).

In an exemplary embodiment, binding domain B1 comprises:
the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1132/1133 (SEQ ID NOs: 73, 74 and 75 and/or SEQ ID NOs: 90, 91, and 92), or the exemplary heavy and light chain variable regions (SEQ ID NO: 3 and SEQ ID NO: 1), or heavy and light antibody chains, which comprise said CDRs, as detailed above.

In a further exemplary embodiment, binding domain B1 comprises:
the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody G12 (SEQ ID NOs: 81, 82 and 83 and/or SEQ ID NOs: 96, 97 and 98), or the exemplary heavy and light chain variable regions (SEQ ID NO: 19 and SEQ ID NO: 17), or heavy and light antibody chains, which comprise said CDRs, as detailed above.

In a further exemplary embodiment, binding domain B1 comprises:
the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody G12_mut (SEQ ID NOs: 81, 82 and 83 and/or SEQ ID NOs: 96, 97 and 98), or the exemplary heavy and light chain variable regions (SEQ ID NO: 29 and SEQ ID NO: 17), or heavy and light antibody chains, which comprise said CDRs, as detailed above.

In a further, and preferred, exemplary embodiment, binding domain B1 comprises:
the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody ffAC_05337 (SEQ ID NOs: 81, 82 and 83 and/or SEQ ID NOs: 96, 97 and 98), or the exemplary heavy and light chain variable regions (SEQ ID NO: 431 and SEQ ID NO: 430), or heavy and light antibody chains, which comprise said CDRs, as detailed above.

The numbering of the antibody (e.g. Antibody X/Y) defines the heavy chain variable region (X) and the light chain variable region (Y), respectively (or, where a single number is indicated, the heavy chain variable region [X] only is defined).

It will be appreciated by persons skilled in the art that the bispecific polypeptides of the invention may alternatively comprise variants of the above-defined variable regions (or variants of the CDR sequences of the B1 and/or B2 binding domains).

A variant of any one of the heavy or light chain amino acid sequences or CDR sequences recited herein may be a substitution, deletion or addition variant of said sequence. A variant may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30 or more amino acid substitutions and/or deletions from the said sequence. "Deletion" variants may comprise the deletion of individual amino acids, deletion of small groups of amino acids

such as 2, 3, 4 or 5 amino acids, or deletion of larger amino acid regions, such as the deletion of specific amino acid domains or other features. "Substitution" variants preferably involve the replacement of one or more amino acids with the same number of amino acids and making conservative amino acid substitutions. For example, an amino acid may be substituted with an alternative amino acid having similar properties, for example, another basic amino acid, another acidic amino acid, another neutral amino acid, another charged amino acid, another hydrophilic amino acid, another hydrophobic amino acid, another polar amino acid, another aromatic amino acid or another aliphatic amino acid. Some properties of the 20 main amino acids which can be used to select suitable substituents are as follows:

Ala, A	aliphatic, hydrophobic, neutral	Met, M	hydrophobic, neutral
Cys, C	polar, hydrophobic, neutral	Asn, N	polar, hydrophilic, neutral
Asp, D	polar, hydrophilic, charged (-)	Pro, P	hydrophobic, neutral
Glu, E	polar, hydrophilic, charged (-)	Gln, Q	polar, hydrophilic, neutral
Phe, F	aromatic, hydrophobic, neutral	Arg, R	polar, hydrophilic, charged (+)
Gly, G	aliphatic, neutral	Ser, S	polar, hydrophilic, neutral
His, H	aromatic, polar, hydrophilic, charged (+)	Thr, T	polar, hydrophilic, neutral
Ile, I	aliphatic, hydrophobic, neutral	Val, V	aliphatic, hydrophobic, neutral
Lys, K	polar, hydrophilic, charged (+)	Trp, W	aromatic, hydrophobic, neutral
Leu, L	aliphatic, hydrophobic, neutral	Tyr, Y	aromatic, polar, hydrophobic

Amino acids herein may be referred to by full name, three letter code or single letter code.

Preferred "derivatives" or "variants" include those in which instead of the naturally occurring amino acid the amino acid which appears in the sequence is a structural analog thereof. Amino acids used in the sequences may also be derivatised or modified, e.g. labelled, providing the function of the polypeptide is not significantly adversely affected.

Derivatives and variants as described above may be prepared during synthesis of the polypeptide or by post-production modification, or when the polypeptide is in recombinant form using the known techniques of site-directed mutagenesis, random mutagenesis, or enzymatic cleavage and/or ligation of nucleic acids.

Preferably variants have an amino acid sequence which has more than 60%, or more than 70%, e.g. 75 or 80%, preferably more than 85%, e.g. more than 90 or 95% amino acid identity to a sequence as shown in the sequences disclosed herein. This level of amino acid identity may be seen across the full length of the relevant SEQ ID NO sequence or over a part of the sequence, such as across 20, 30, 50, 75, 100, 150, 200 or more amino acids, depending on the size of the full-length polypeptide.

In connection with amino acid sequences, "sequence identity" refers to sequences which have the stated value when assessed using ClustalW (Thompson et al., 1994; the disclosures of which are incorporated herein by reference) with the following parameters:

Pairwise alignment parameters - Method: accurate, Matrix: PAM, Gap open penalty: 10.00, Gap extension penalty: 0.10.

Multiple alignment parameters - Matrix: PAM, Gap open penalty: 10.00, % identity for delay: 30, Penalize end gaps: on, Gap separation distance: 0, Negative matrix: no, Gap extension penalty: 0.20, Residue-specific gap penalties: on, Hydrophilic gap penalties: on, Hydrophilic residues: GPSNDQEKR. Sequence identity at a particular residue is intended to include identical residues which have simply been derivatised.

In one embodiment, binding domain B1 comprises the light chain of antibody 1132/1133 (SEQ ID NO: 372 or 379) and/or the heavy chain of antibody 1132/1133 (SEQ ID NO: 371 or 378).

In one embodiment, binding domain B1 comprises the light chain of antibody G12 (SEQ ID NO: 381) and/or the heavy chain of antibody G12 (SEQ ID NO: 380).

In one embodiment, binding domain B1 comprises the light chain of antibody G12_mut (SEQ ID NO: 383) and/or the heavy chain of antibody G12_mut (SEQ ID NO: 382).

It will be appreciated by the skilled person, and it is included herein, that mutations described herein for the RUBY™ format and/or the optimised RUBY™ format can be applied to the above light chain and/or the heavy chain sequences of G12 and/or G12_mut.

Thus, in one embodiment binding domain B1 may comprise one or more variants of the above-defined light chain variable regions and/or said heavy chain variable regions

(and/or light chain and/or said heavy chain) having at least 90% sequence identity thereto or 95% sequence identity thereto or 99% sequence identity thereto. Binding domain B1 may also comprise variants of the CDR sequences specified herein, for example variants where up one, two, three, four or five amino acid residues are substituted, deleted to added compared to the specified reference sequences.

For reference, the antibody reference used in this application, possible alternative names for the same antibody/binding domain, and the target of the antibody/binding domain, is laid out in Table i below.

Table i: Alternative names for particular CD40 antibodies/binding domains

Antibody reference	**Alternative names**
1132	1132/1133
1150	1150/1151
1140	1140/1135
1107	1107/1108
G12	ADC-1013
APX005	
21.4.1	
G12_mut	

The "G12_mut" antibody largely corresponds to the sequence of "G12"; however, G12_mut includes three mutations in the VH framework . The CDRs and the VL sequences of G12_mut are the same as the G12.

Accordingly, in a further independent aspect of the present invention is a polypeptide which is capable of binding specifically to CD40 comprising a heavy variable region comprising SEQ ID NO: 29 and/or a light chain variable region comprising SEQ ID NO: 17, preferably wherein the polypeptide is monospecific, further preferably wherein the polypeptide is an agonistic CD40 polypeptide, further preferably wherein the light chain comprises SEQ ID NO: 383 and/or the heavy chain comprises SEQ ID NO: 382, and further preferably wherein the polypeptide is the antibody G12_mut.

Embodiments of the other aspects of the invention (such as aspect one and/or aspect two) can be incorporated into this aspect of the invention, of the polypeptide capable of binding specifically to CD40 comprising a heavy variable region comprising SEQ ID NO: 29 and/or a light chain variable region comprising SEQ ID NO: 17.

Carcinoembryonic antigen (CEA) binders

The bispecific polypeptides of the invention further comprise a binding domain (B2) which is capable of specifically binding a carcinoembryonic antigen (CEA).

Binding domain B2 specifically binds to CEA, *i.e.* it binds to CEA but does not bind, or binds at a lower affinity, to other molecules. The term CEA, as used herein, typically refers to human CEA. Binding domain B2 may have some binding affinity for CEA from other mammals, such as CEA from a non-human primate (for example *Macaca fascicularis* (cynomolgus monkey), *Macaca mulatta*). Binding domain B2 preferably does not bind to non-target molecules, such as CTLA-4-Fc and/or human ubiquitin.

In one embodiment, the CEA is a tumor-associated CEA. By "tumor-associated CEA" we include a member of the CEA family whose presence and/or overexpression is correlated with the existence of cancer and/or tumours; for example, a CEA that is known or suspected to be overexpressed by cancer and/or tumour cells. Members of the CEA family that are associated with tumours and/or cancer would be known to the skilled person; for example, CEACAM1, CEACAM6, CEACAM7 and/or CEACAM5.

In one embodiment, the CEA is a carcinoembryonic antigen-related cell adhesion molecule (CEACAM).

In one embodiment, the CEACAM is one or more selected from the listing consisting of: CEACAM1 (such as, GenBank: NG_029051.2); CEACAM3 (such as, GenBank: D90278.1); CEACAM4 (such as, GenBank: D90276.1); CEACAM5 (such as, GenBank: M17303.1); CEACAM6 (such as, GenBank: M29541.1); CEACAM7 (such as, GenBank: L31792.1); CEACAM8 (such as, GenBank: X52378.1); CEACAM16 (such as, GenBank: EU021223.1); CEACAM18 (such as, GenBank: AC020914.9); CEACAM19 (such as, GenBank: BC083499.1); CEACAM20 (such as, GenBank: AY358129.1); and CEACAM21 (such as, GenBank: BC106727.1). It will be appreciated that the reference to the aforementioned CEACAM molecules includes splice variants.

Preferably, the CEACAM is one or more selected from the listing consisting of: CEACAM1; CEACAM5; and CEACAM6. Preferably, the CEACAM is CEACAM1. Most preferably, the CEACAM is CEACAM5.
In a preferred embodiment, B2 is capable of specifically binding to CEACAM5 but not other CEACAMs, particularly not CEACAM1.

In one embodiment, B2 which is capable of specifically binding to CEA on a target cell. Preferably, the target cell is a cancer cell and/or a tumour cell.

Preferably, the CEA on the target cell is an intermediate level of CEA or a high level of CEA.

In one embodiment, the intermediate level of CEA expression is characterised by the target cell expressing about 10,000 or more CEA receptors per target cell; for example, about 11,000 or more; about 12,000 or more; about 13,000 or more; about 14,000 or more; about 15,000 or more; about 16,000 or more; about 17,000 or more; about 18,000 or more; about 19,000 or more; about 20,000 or more; about 25,000 or more; about 30,000 or more; about 35,000 or more; about 40,000 or more; about 50,000 or more; about 60,000 or more; about 70,000 or more; about 80,000 or more; about 90,000 or more; about 100,000 or more; about 125,000 or more; about 150,000 or more; or about 175,000 or more CEA receptors per target cell. In another embodiment, the intermediate level of CEA expression is characterised by the target cell expressing about 10,000 to about 200,000 CEA receptors per target cell; for example, about 20,000 to about 175,000 CEA receptors per target cell or 20,000 to about 200,000 CEA receptors per target cell or about 50,000 to about 175,000 CEA receptors per target cell or about 50,000 to about 200,000 CEA receptors per target cell. Preferably, the CEA receptors are CEACAM5 receptors.

In one embodiment, the high level of CEA expression is characterised by the target cell expressing about 200,000 or more CEA receptors per target cell; for example, about 225,000 or more; about 250,000 or more; about 275,000 or more; about 300,000 or more; about 325,000 or more; about 350,000 or more; about 375,000 or more; about 400,000 or more; about 425,000 or more; about 450,000 or more; about 475,000 or more; about 500,000 or more; about 600,000 or more; about 700,000 or more; about 800,000 or more; about 900,000 or more; or about 1,000,000 CEA receptors per target cell, preferably about 300,000 of more CEA receptors per target cell. In another embodiment, the high level of CEA expression is characterised by the target cell expressing about 200,000 to about 1,000,000 CEA receptors per target cell; for example, about 200,000 to about 500,000 CEA receptors per target cell or about 300,000 to about 500,000 CEA receptors per target cell. Preferably, the CEA receptors are CEACAM5 receptors.

In one embodiment, B2 is not capable of specifically binding to a cell with no CEA expression or a low level of CEA expression. In one embodiment, the low level of CEA expression is characterised by a cell expressing about 10,000 or fewer CEA receptors per cell; for example, about 9,000 or fewer; about 8,000 or fewer; about 7,000 or fewer; about 6,000 or fewer; about 5,000 or fewer; about 4,000 or fewer; about 3,000 or fewer; about 2,000 or fewer; or about 1,000 or fewer CEA receptors per cell.

Advantageously, binding domain B2 binds to human CEA with a K_D of less than $2x10^{-6}$M or less than $1.5x10^{-8}$M or less than $2.5x10^{-9}$M or less than $2x10^{-9}$M or less than $1.5x10^{-12}$M or less than $1x10^{-12}$M, preferably less than $1.5x10^{-8}$M or less than $2.5x10^{-9}$M or less than $1.5x10^{-12}$M. Preferably, the K_D is measured in Octet; for example, as explained in the Examples.

For example, binding domain B2 preferably does not bind to non-target molecules, such as CTLA-4-Fc and/or human ubiquitin. In a particular embodiment relating to a specific CEACAM, the non-target molecule may be a different CEACAM; for example, for CEACAM5 the non-target molecule may be CEACAM, and *vice versa*. Therefore, typically, the K_D for the binding domain with respect to human CEA will be 2-fold, preferably 5-fold, more preferably 10-fold less than K_D with respect to the other, non-target molecules, such as CTLA-4-Fc and/or human ubiquitin or any other unrelated material or accompanying material in the environment. More preferably, the K_D will be 50-fold less, even more preferably 100-fold less, and yet more preferably 200-fold less.

Binding domain B2 is preferably capable of binding to its target with an affinity that is at least two-fold, 10-fold, 50-fold, 100-fold or greater than its affinity for binding to another non-target molecule.

In summary therefore, binding domain B2 preferably exhibits at least one of the following functional characteristics:

a) binding to human CEA with a K_D value which is less than $2x10^{-6}$M, more preferably less than $2.5x10^{-9}$M or less than $1.5x10^{-12}$M, more preferably less than $1.5x10^{-12}$M;

b) does not bind to non-target molecules, such as CTLA-4-Fc and/or human ubiquitin.

In one embodiment, binding domain B2 binds preferentially to CEA on a cell over soluble CEA. By "binds preferentially to CEA on a cell over soluble CEA", we include that when in the presence of CEA on a cell (such as, on the surface of a cell) and soluble CEA, B2 will be more likely to bind to CEA on the cell than the soluble CEA.

In one embodiment, binding domain B2 comprises one or more light chain CDR sequences selected from those in Table D(2) and/or one or more heavy chain CDR sequences selected from Table D(1a) and/or Table D(1b). Thus binding domain B2 may comprise one or more CDR sequences selected from the groups consisting of:

(a) CEA heavy chain CDRs, SEQ ID NOs: 216 to 310, 335; and/or

(b) CEA light chain CDRs, SEQ ID NOs: 90, 91, 94, 311 to 334.

In one embodiment binding domain B2 comprises one, two or three light chain CDR sequences from a particular row for an individual antibody reference in Table D(2), and/or one, two or three heavy chain CDR sequences from the corresponding row for the antibody with the same reference in Table D(1a) and/or Table D(1b). For example, binding domain B2 might comprise one or more of the light chain CDR sequences for AC_05059 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05059 (SEQ ID NOs: 216, 217, and 218 or 280, 281 and 218) or one or more of the light chain CDR sequences for AC_05060 (SEQ ID NOs: 312, 91, and 313) and one or more of the heavy chain CDR sequences for AC_05060 (SEQ ID NOs: 219, 220, and 221 or 282, 283 and 221) or one or more of the light chain CDR sequences for AC_05061 (SEQ ID NOs: 90, 91 and 314) and one or more of the heavy chain CDR sequences for AC_05061 (SEQ ID NOs: 222, 223 and 224 or 284, 285 and 224) or one or more of the light chain CDR sequences for AC_05062 (SEQ ID NOs: 315, 316 and 94) and one or more of the heavy chain CDR sequences for AC_05062 (SEQ ID NOs: 222, 223 and 225 or 284, 285 and 225) or one or more of the light chain CDR sequences for AC_05064 (SEQ ID NOs: 90, 91 and 317) and one or more of the heavy chain CDR sequences for AC_05064 (SEQ ID NOs: 222, 223 and 226 or 284, 285 and 226) or one or more of the light chain CDR sequences for AC_05079 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05079 (SEQ ID NOs: 216, 217 and 227 or 280, 281 and 227) or one or more of the light chain CDR sequences for AC_05081 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05081 (SEQ ID NOs: 216, 217 and 229 or 280, 281 and 229) or one or more of the light chain CDR sequences for AC_05088 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05088 (SEQ ID NOs: 216, 217 and 237 or 280, 281 and 237) or one or more of the light chain CDR sequences for AC_05089 (SEQ ID NOs: 90, 91 and 311)

and one or more of the heavy chain CDR sequences for AC_05089 (SEQ ID NOs: 216, 217, and 238 or 280, 281 and 238) or one or more of the light chain CDR sequences for AC_05090 (SEQ ID NOs: 90, 91, and 311) and one or more of the heavy chain CDR sequences for AC_05090 or ffAC_05337 (SEQ ID NOs: 216, 217 and 239 or 280, 281 and 239) or one or more of the light chain CDR sequences for AC_05091 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05091 (SEQ ID NOs: 216, 217 and 240 or 280, 281 and 240) or one or more of the light chain CDR sequences for AC_05093 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05093 (SEQ ID NOs: 216, 217 and 241 or 280, 281 and 241) or one or more of the light chain CDR sequences for AC_05094 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05094 (SEQ ID NOs: 216, 217 and 242 or 280, 281 and 242) or one or more of the light chain CDR sequences for AC_05096 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05096 (SEQ ID NOs: 216, 217 and 244 or 280, 281 and 244) or one or more of the light chain CDR sequences for AC_05097 (SEQ ID NOs: 90, 91 and 311) and one or more of the heavy chain CDR sequences for AC_05097 (SEQ ID NOs: 216, 217 and 245 or 280, 281 and 245) or one or more of the light chain CDR sequences for Fab1 (SEQ ID NOs: 90, 91 and 322) and one or more of the heavy chain CDR sequences for Fab1 (SEQ ID NOs: 248, 249 and 250 or 289, 290 and 250) or one or more of the light chain CDR sequences for Fab3 (SEQ ID NOs: 324, 325 and 326) and one or more of the heavy chain CDR sequences for Fab3 (SEQ ID NOs: 254, 255 and 256 or 293, 294 and 256). Most preferably, B2 comprises the CDRs and/or the VL and VH of AC_05088, AC_05090/ffAC_05337, AC_05093, AC_05097, Fab1, and/or Fab3.

As explained further in the Examples, the references to exemplary B2 polypeptides (such as "Fab1") are nomenclature based on the libraries from which the particular binders were identified, and are not specific references to particular types, or fragments, of antibodies. To put another way, "Fab1" is not necessarily a Fab fragment. Accordingly, the CDRs, VL and VH amino acid sequences defined for each of the exemplary B2 polypeptides can be used in any compatible antibody format, or fragment thereof.

Preferred CEA binding domains may comprise at least a heavy chain CDR3 as defined in any individual row of Table D(1a) and/or a light chain CDR3 as defined in in any individual row of Table D(2).

Accordingly, in one embodiment binding domain B2 comprises all six CDR sequences for a given antibody (VH/VL) reference, for example binding domain B2 might comprise all six CDR sequences of an antibody selected from the list consisting of: AC_05059; AC_05060; AC_05061; AC_05062; AC_05064; AC_05079; AC_05080; AC_05081; AC_05082; AC_05083; AC_05084; AC_05085; AC_05086; AC_05087; AC_05088; AC_05089; AC_05090; AC_05091; AC_05092; AC_05093; AC_05094; AC_05095; AC_05096; AC_05097; AC_05098; AC_05099; AC_05100; Fab1; Fab2; Fab3; Fab4; Fab5; Fab6; Fab7; Fab8; Fab9; Fab10; Fab11; ffAC_05337 and mAb2, preferably: AC_05059; AC_05060; AC_05061; AC_05062; AC_05064; AC_05079; AC_05081; AC_05088; AC_05089; AC_05090; AC_05091; AC_05093; AC_05094; AC_05096; AC_05097; Fab1; ffAC_05337 and Fab3, most preferably AC_05088; AC_05090; the CEA binding domain of ffAC_05337; AC_05093; AC_05097; Fab1; and Fab3.

In one embodiment, binding domain B2 comprises a VH and/or a VL amino acid sequence as given in Table B. In one embodiment, binding domain B2 comprises a VH and VL amino acid sequence as given in Table B for a particular antibody reference. For example, binding domain B2 may comprise the VH sequence of AC_05059 (SEQ ID NO: 33) and/or the VL sequence of AC_05059 (SEQ ID NO: 31) or binding domain B2 may comprise the VH sequence of AC_05060 (SEQ ID NO: 37) and/or the VL sequence of AC_05060 (SEQ ID NO: 35) or binding domain B2 may comprise the VH sequence of AC_05062 (SEQ ID NO: 45) and/or the VL sequence of AC_05062 (SEQ ID NO: 43) or binding domain B2 may comprise the VH sequence of AC_05064 (SEQ ID NO: 49) and/or the VL sequence of AC_05064 (SEQ ID NO: 47) or binding domain B2 may comprise the VH sequence of AC_05079 (SEQ ID NO: 53) and/or the VL sequence of AC_05079 (SEQ ID NO: 51) or binding domain B2 may comprise the VH sequence of AC_05081 (SEQ ID NO: 61) and/or the VL sequence of AC_05081 (SEQ ID NO: 59) or binding domain B2 may comprise the VH sequence of AC_05088 (SEQ ID NO: 122) and/or the VL sequence of AC_05088 (SEQ ID NO: 120) or binding domain B2 may comprise the VH sequence of AC_05089 (SEQ ID NO: 126) and/or the VL sequence of AC_05089 (SEQ ID NO: 124) or binding domain B2 may comprise the VH sequence of AC_05090 (SEQ ID NO: 130) and/or the VL sequence of AC_05090 (SEQ ID NO: 128) or binding domain B2 may comprise the VH sequence of AC_05091 (SEQ ID NO: 134) and/or the VL sequence of AC_05091 (SEQ ID NO: 132) or binding domain B2 may comprise the VH sequence of AC_05093 (SEQ ID NO: 142) and/or the VL sequence of AC_05093 (SEQ ID NO: 140) or binding domain B2 may comprise the VH sequence of AC_05094 (SEQ ID NO: 146) and/or the VL sequence of AC_05094 (SEQ ID NO: 144) or binding domain B2 may comprise the VH sequence of AC_05096 (SEQ ID NO: 154) and/or the VL sequence of AC_05096 (SEQ ID NO: 152) or binding domain B2 may

comprise the VH sequence of AC_05097 (SEQ ID NO: 158) and/or the VL sequence of AC_05097 (SEQ ID NO: 156) or binding domain B2 may comprise the VH sequence of Fab1 (SEQ ID NO: 174) and/or the VL sequence of Fab1 (SEQ ID NO: 172) or binding domain B2 may comprise the VH sequence of Fab3 (SEQ ID NO: 182) and/or the VL sequence of Fab3 (SEQ ID NO: 180) or binding domain B2 may comprise the VH sequence of ffAC_05337 (SEQ ID NO: 433) and/or the VL sequence of ffAC_05337 (SEQ ID NO: 432).

In one embodiment the CEA binding domain of B2 is selected from: AC_05059; AC_05060; AC_05061; AC_05062; AC_05064; AC_05079; AC_05080; AC_05081; AC_05082; AC_05083; AC_05084; AC_05085; AC_05086; AC_05087; AC_05088; AC_05089; AC_05090; AC_05091; AC_05092; AC_05093; AC_05094; AC_05095; AC_05096; AC_05097; AC_05098; AC_05099; AC_05100; Fab1; Fab2; Fab3; Fab4; Fab5; Fab6; Fab7; Fab8; Fab9; Fab10; Fab11; the CEA binding domain of ffAC_05337 and mAb2, preferably: AC_05059; AC_05060; AC_05061; AC_05062; AC_05064; AC_05079; AC_05081; AC_05088; AC_05089; AC_05090; AC_05091; AC_05093; AC_05094; AC_05096; AC_05097; Fab1; the CEA binding domain of ffAC_05337; and Fab3, most preferably AC_05088; AC_05090; the CEA binding domain of ffAC_05337; AC_05093; AC_05097; Fab1; and Fab3.

Thus, the CDR or VH and VL sequences of binding domain B2 might be selected from antibodies from the group consisting of:

(a) AC_05059 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 218 or 280, 281 and 218; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 31; VH: SEQ ID NO: 33)

(b) AC_05060 (heavy chain CDRs: SEQ ID NOs: 219, 220 and 221 or 282, 283 and 221; light chain CDRs: SEQ ID NOs: 312, 91 and 313; VL: SEQ ID NO: 35; VH: SEQ ID NO: 37)

(c) AC_05061 (heavy chain CDRs: SEQ ID NOs: 222, 223 and 224 or 284, 285 and 224; light chain CDRs: SEQ ID NOs: 90, 91 and 314; VL: SEQ ID NO: 39; VH: SEQ ID NO: 41)

(d) AC_05062 (heavy chain CDRs: SEQ ID NOs: 222, 223 and 225 or 284, 285 and 225; light chain CDRs: SEQ ID NOs: 315, 316 and 94; VL: SEQ ID NO: 43; VH: SEQ ID NO: 45)

(e) AC_05064 (heavy chain CDRs: SEQ ID NOs: 222, 223 and 226 or 284, 285 and 226; light chain CDRs: SEQ ID NOs: 90, 91 and 317; VL: SEQ ID NO: 47; VH: SEQ ID NO: 49)

(f) AC_05079 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 227 or 280, 281 and 227; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 51; VH: SEQ ID NO: 53)

(g) AC_05080 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 228 or 280, 281 and 228; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 55; VH: SEQ ID NO: 57)

(h) AC_05081 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 229 or 280, 281 and 229; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 59; VH: SEQ ID NO: 61)

(i) AC_05082 (heavy chain CDRs: SEQ ID NOs: 222, 223 and 230 or 284, 285 and 230; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 63; VH: SEQ ID NO: 65)

(j) AC_05083 (heavy chain CDRs: SEQ ID NOs: 222, 223 and 231 or 284, 285 and 231; light chain CDRs: SEQ ID NOs: 318, 91 and 319; VL: SEQ ID NO: 67; VH: SEQ ID NO: 69)

(k) AC_05084 (heavy chain CDRs: SEQ ID NOs: 222, 223 and 232 or 284, 285 and 232; light chain CDRs: SEQ ID NOs: 90, 91 and 320; VL: SEQ ID NO: 71; VH: SEQ ID NO: 106)

(l) AC_05085 (heavy chain CDRs: SEQ ID NOs: 219, 233 and 234 or 286, 287 and 234; light chain CDRs: SEQ ID NOs: 90, 91 and 311 ; VL: SEQ ID NO: 108; VH: SEQ ID NO: 110)

(m) AC_05086 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 235 or 280, 281 and 235; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 112; VH: SEQ ID NO: 114)

(n) AC_05087 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 236 or 280, 281 and 236; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 116; VH: SEQ ID NO: 118)

(o) AC_05088 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 237 or 280, 281 and 237; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 120; VH: SEQ ID NO: 122)

(p) AC_05089 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 238 or 280, 281 and 238; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 124; VH: SEQ ID NO: 126)

(q) AC_05090 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 239 or 280, 281 and 239; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 128; VH: SEQ ID NO: 130)

(r) AC_05091 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 240 or 280, 281 and 240; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 132; VH: SEQ ID NO: 134)

(s) AC_05092 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 218 or 280, 281 and 218; light chain CDRs: SEQ ID NOs: 321, 91 and 311; VL: SEQ ID NO: 136; VH: SEQ ID NO: 138)

(t) AC_05093 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 241 or 280, 281 and 241; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 140; VH: SEQ ID NO: 142)

(u) AC_05094 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 242 or 280, 281 and 242; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 144; VH: SEQ ID NO: 146)

(v) AC_05095 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 243 or 280, 281 and 243; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 148; VH: SEQ ID NO: 150)

(w) AC_05096 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 244 or 280, 281 and 244; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 152; VH: SEQ ID NO: 154)

(x) AC_05097 (heavy chain CDRs: SEQ ID NOs: 217, 216 and 245 or 280, 281 and 245; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 156; VH: SEQ ID NO: 158)

(y) AC_05098 (heavy chain CDRs: SEQ ID NOs: 219, 220 and 246 or 282, 283 and 246; light chain CDRs: SEQ ID NOs: 312, 91 and 313; VL: SEQ ID NO: 160; VH: SEQ ID NO: 162)

(z) AC_05099 (heavy chain CDRs: SEQ ID NOs: 222, 223 and 224 or 288, 285 and 224; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 164; VH: SEQ ID NO: 166)

(aa) AC_05100 (heavy chain CDRs: SEQ ID NOs: 222, 223 and 247 or 288, 285 and 247; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 168; VH: SEQ ID NO: 170)

(ab) Fab1 (heavy chain CDRs: SEQ ID NOs: 248, 249 and 250 or 289, 290 and 250; light chain CDRs: SEQ ID NOs: 90, 91 and 322; VL: SEQ ID NO: 172; VH: SEQ ID NO: 174)

(ac) Fab2 (heavy chain CDRs: SEQ ID NOs: 251, 252 and 253 or 291, 292 and 253; light chain CDRs: SEQ ID NOs: 90, 91 and 323; VL: SEQ ID NO: 176; VH: SEQ ID NO: 178)

(ad) Fab3 (heavy chain CDRs: SEQ ID NOs: 254, 255 and 256 or 293, 294 and 256; light chain CDRs: SEQ ID NOs: 324, 325 and 326; VL: SEQ ID NO: 180; VH: SEQ ID NO: 182)

(ae) Fab4 (heavy chain CDRs: SEQ ID NOs: 257, 258 and 259 or 295, 296 and 259; light chain CDRs: SEQ ID NOs: 90, 91 and 327; VL: SEQ ID NO: 184; VH: SEQ ID NO: 186)

(af) Fab5 (heavy chain CDRs: SEQ ID NOs: 260, 261 and 262 or 297, 298 and 262; light chain CDRs: SEQ ID NOs: 324, 325 and 328; VL: SEQ ID NO: 188; VH: SEQ ID NO: 190)

(ag) Fab6 (heavy chain CDRs: SEQ ID NOs: 263, 264 and 265 or 299, 300 and 265; light chain CDRs: SEQ ID NOs: 324, 325 and 329; VL: SEQ ID NO: 192; VH: SEQ ID NO: 194)

(ah) Fab7 (heavy chain CDRs: SEQ ID NOs: 266, 267 and 268 or 301, 302 and 268; light chain CDRs: SEQ ID NOs: 90, 91 and 330; VL: SEQ ID NO: 196; VH: SEQ ID NO: 198)

(ai) Fab8 (heavy chain CDRs: SEQ ID NOs: 269, 270 and 271 or 303, 304 and 271; light chain CDRs: SEQ ID NOs: 90, 91 and 331; VL: SEQ ID NO: 200; VH: SEQ ID NO: 202)

(aj) Fab9 (heavy chain CDRs: SEQ ID NOs: 272, 335 and 273 or 305, 306 and 273; light chain CDRs: SEQ ID NOs: 90, 91 and 332; VL: SEQ ID NO: 204; VH: SEQ ID NO: 206)

(ak) Fab10 (heavy chain CDRs: SEQ ID NOs: 274, 275 and 276 or 307, 308 and 276; light chain CDRs: SEQ ID NOs: 90, 91 and 333; VL: SEQ ID NO: 208; VH: SEQ ID NO: 210)

(al) Fab11 (heavy chain CDRs: SEQ ID NOs: 277, 278 and 279 or 309, 310 and 279; light chain CDRs: SEQ ID NOs: 324, 325 and 334; VL: SEQ ID NO: 212; VH: SEQ ID NO: 214) and/or

(am) ffAC_05337 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 239; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 432; VH: SEQ ID NO: 433), preferably (am) ffAC_05337 (heavy chain CDRs: SEQ ID NOs: 216, 217 and 239; light chain CDRs: SEQ ID NOs: 90, 91 and 311; VL: SEQ ID NO: 432; VH: SEQ ID NO: 433),.

The numbering of the antibody (e.g. Antibody X/Y) defines the heavy chain variable region (X) and the light chain variable region (Y), respectively (or, where a single number is indicated, the heavy chain variable region [X] only is defined). As described above, the sequences may be one or more CDR sequence, or the VH and/or VL

sequence. As described above, the sequences of the bispecific polypeptide may comprise specified mutations.

A variant of any one of the heavy or light chain amino acid sequences or CDR sequences recited herein may be a substitution, deletion or addition variant of said sequence. A variant may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30 or more amino acid substitutions and/or deletions from the said sequence. "Deletion" variants may comprise the deletion of individual amino acids, deletion of small groups of amino acids such as 2, 3, 4 or 5 amino acids, or deletion of larger amino acid regions, such as the deletion of specific amino acid domains or other features. "Substitution" variants preferably involve the replacement of one or more amino acids with the same number of amino acids and making conservative amino acid substitutions. For example, an amino acid may be substituted with an alternative amino acid having similar properties, for example, another basic amino acid, another acidic amino acid, another neutral amino acid, another charged amino acid, another hydrophilic amino acid, another hydrophobic amino acid, another polar amino acid, another aromatic amino acid or another aliphatic amino acid. Some properties of the 20 main amino acids which can be used to select suitable substituents are as follows:

Ala, A	aliphatic, hydrophobic, neutral	Met, M	hydrophobic, neutral
Cys, C	polar, hydrophobic, neutral	Asn, N	polar, hydrophilic, neutral
Asp, D	polar, hydrophilic, charged (-)	Pro, P	hydrophobic, neutral
Glu, E	polar, hydrophilic, charged (-)	Gln, Q	polar, hydrophilic, neutral
Phe, F	aromatic, hydrophobic, neutral	Arg, R	polar, hydrophilic, charged (+)
Gly, G	aliphatic, neutral	Ser, S	polar, hydrophilic, neutral
His, H	aromatic, polar, hydrophilic, charged (+)	Thr, T	polar, hydrophilic, neutral
Ile, I	aliphatic, hydrophobic, neutral	Val, V	aliphatic, hydrophobic, neutral
Lys, K	polar, hydrophilic, charged (+)	Trp, W	aromatic, hydrophobic, neutral
Leu, L	aliphatic, hydrophobic, neutral	Tyr, Y	aromatic, polar, hydrophobic

Amino acids herein may be referred to by full name, three letter code or single letter code.

Preferred "derivatives" or "variants" include those in which instead of the naturally occurring amino acid the amino acid which appears in the sequence is a structural analog thereof. Amino acids used in the sequences may also be derivatised or modified,

e.g. labelled, providing the function of the polypeptide is not significantly adversely affected.

Derivatives and variants as described above may be prepared during synthesis of the polypeptide or by post-production modification, or when the polypeptide is in recombinant form using the known techniques of site-directed mutagenesis, random mutagenesis, or enzymatic cleavage and/or ligation of nucleic acids.

Preferably variants have an amino acid sequence which has more than 60%, or more than 70%, e.g. 75 or 80%, preferably more than 85%, e.g. more than 90 or 95% amino acid identity to a sequence as shown in the sequences disclosed herein. This level of amino acid identity may be seen across the full length of the relevant SEQ ID NO sequence or over a part of the sequence, such as across 20, 30, 50, 75, 100, 150, 200 or more amino acids, depending on the size of the full-length polypeptide.

In one embodiment binding domain B2 is specific for CEA, typically human CEA and may comprise any one, two, three, four, five or all six features independently selected from the following:

(a) a heavy chain CDR1 sequence which consists of the sequence: "G, F, T, F, S, S, S, Y" or which comprises the consensus sequence of: "G, F, T, F, G/S, S, Y, Y/A";

(b) a heavy chain CDR2 sequence which consists of the sequence: "I, G, S, G, S, Y, S, T" or which comprises the consensus sequence of: "I, S, G, Y/S, G, Y/G, S, T";

(c) a heavy chain CDR3 sequence which comprises the consensus sequence of: "A, R, Y, P, S, V, P/L, F, P, Q, S, P/H/L, H/P/L, L/F/V/W, D, Y" or which comprises the consensus sequence of: "A, R, H/Y, G, Y, G/S/T, V/ H, L/F, D, Y";

(d) a light chain CDR1 sequence which consists of the sequence: "Q, S, I, S, S, Y" or which comprises the consensus sequence of: "Q, S, I, R/S, S, Y";

(e) a light chain CDR2 sequence which consists of the sequence: "A, A, S";

(f) a light chain CDR3 sequence which consists of the sequence: "Q, Q, A, G, N, P, H, T" or which comprises the consensus sequence of: "Q, Q, G/Y, T/P/A, W/-, Y/-, F/V, P, F/Y, T".

In an alternative embodiment binding domain B2 is specific for CEA, typically human CEA and may comprise any one, two, three, four, five or all six features independently selected from the following:

(a) a heavy chain CDR1 sequence which consists of the sequence: "G, F, T, F, S, S, S, Y";

(b) a heavy chain CDR2 sequence which consists of the sequence: "I, G, S, G, S, Y, S, T";

(c) a heavy chain CDR3 sequence which comprises the consensus sequence of: "A, R, Y, P, S, V, P/L, F, P, Q, S, P/H/L, H/P/L, L/F/V/W, D, Y";

(d) a light chain CDR1 sequence which consists of the sequence: "Q, S, I, S, S, Y";

(e) a light chain CDR2 sequence which consists of the sequence: "A, A, S";

(f) a light chain CDR3 sequence which consists of the sequence: "Q, Q, A, G, N, P, H, T". Binding domain B2 may comprise at least a heavy chain CDR3 as defined in (c) of this embodiment and/or a light chain CDR3 as defined in (f). Binding domain B2 may comprise all three heavy chain CDR sequences of (a), (b) and (c) and/or all three light chain CDR sequences of (d), (e) and (f).

In a further alternative embodiment binding domain B2 is specific for CEA, typically human CEA and may comprise any one, two, three, four, five or all six features independently selected from the following:

(a) a heavy chain CDR1 sequence which comprises the consensus sequence of: "G, F, T, F, G/S, S, Y, Y/A";

(b) a heavy chain CDR2 sequence which comprises the consensus sequence of: "I, S, G, Y/S, G, Y/G, S, T";

(c) a heavy chain CDR3 sequence which comprises the consensus sequence of: "A, R, H/Y, G, Y, G/S/T, V/ H, L/F, D, Y";

(d) a light chain CDR1 sequence which comprises the consensus sequence of: "Q, S, I, R/S, S, Y";

(e) a light chain CDR2 sequence which consists of the sequence: "A, A, S";

(f) a light chain CDR3 sequence which comprises the consensus sequence of: "Q, Q, G/Y, T/P/A, W/-, Y/-, F/V, P, F/Y, T".

Binding domain B2 may comprise at least a heavy chain CDR3 as defined in (c) of this embodiment and/or a light chain CDR3 as defined in (f). Binding domain B2 may comprise all three heavy chain CDR sequences of (a), (b) and (c) and/or all three light chain CDR sequences of (d), (e) and (f).

Examples of complete heavy and light chain variable region amino acid sequences for binding domain B2 are shown in Table B. Exemplary nucleic acid sequences encoding each amino acid sequence are also shown. The numbering of said VH and VL regions in Table B corresponds to the numbering system used as in Table D(1a), Table D(1b) and/or Table D(2). Thus, for example, the amino acid sequence for "AC_05088, light

chain VL" is an example of a complete VL region sequence comprising all three CDRs of VL number AC_05088 shown in Table D(2)and the amino acid sequence for "AC_05088, heavy chain VH" is an example of a complete VH region sequence comprising all three CDRs of VH number AC_05088 shown in Table D(1a) and/or Table D(1b).

In exemplary embodiments, binding domain B2 comprises:

(a) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05059 (SEQ ID NOs: 216, 217 and 218 or 280, 281 and 218 and/or SEQ ID NOs: 90, 91 and 311)

(b) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05060 (SEQ ID NOs: 219, 220 and 221 or 282, 283 and 221 and/or SEQ ID NOs: 312, 91 and 313)

(c) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05061 (SEQ ID NOs: 222, 223 and 224 or 284, 285 and 224 and/or SEQ ID NOs: 90, 91 and 314)

(d) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05062 (SEQ ID NOs: 222, 223 and 225 or 284, 285 and 225 and/or SEQ ID NOs: 315, 316 and 94)

(e) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05064 (SEQ ID NOs: 222, 223 and 226 or 284, 285 and 226 and/or SEQ ID NOs: 90, 91 and 317)

(f) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05079 (SEQ ID NOs: 216, 217 and 227 or 280, 281 and 227 and/or SEQ ID NOs: 90, 91 and 311)

(g) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05080 (SEQ ID NOs: 216, 217 and 228 or 280, 281 and 228 and/or SEQ ID NOs: 90, 91 and 311)

(h) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05081 (SEQ ID NOs: 216, 217 and 229 or 280, 281 and 229 and/or SEQ ID NOs: 90, 91 and 311)

(i) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05082 (SEQ ID NOs: 222, 223 and 230 or 284, 285 and 230 and/or SEQ ID NOs: 90, 91 and 311)

(j) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05083 (SEQ ID NOs: 222, 223 and 231 or 284, 285 and 231 and/or SEQ ID NOs: 318, 91 and 319)

(k) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05084 (SEQ ID NOs: 222, 223 and 232 or 284, 285 and 232 and/or SEQ ID NOs: 90, 91 and 320)

(l) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05085 (SEQ ID NOs: 219, 233 and 234 or 286, 287 and 234 and/or SEQ ID NOs: 90, 91 and 311)

(m) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05086 (SEQ ID NOs: 216, 217 and 235 or 280, 281 and 235 and/or SEQ ID NOs: 90, 91 and 311)

(n) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05087 (SEQ ID NOs: 216, 217 and 236 or 280, 281 and 236 and/or SEQ ID NOs: 90, 91 and 311)

(o) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05088 (SEQ ID NOs: 216, 217 and 237 or 280, 281 and 237 and/or SEQ ID NOs: 90, 91 and 311)

(p) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05089 (SEQ ID NOs: 216, 217 and 238 or 280, 281 and 238 and/or SEQ ID NOs: 90, 91 and 311)

(q) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05090/ ffAC_05337 (SEQ ID NOs: 216, 217 and 239 or 280, 281 and 239 and/or SEQ ID NOs: 90, 91 and 311)

(r) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05091 (SEQ ID NOs: 216, 217 and 240 or 280, 281 and 240 and/or SEQ ID NOs: 90, 91 and 311)

(s) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05092 (SEQ ID NOs: 216, 217 and 218 or 280, 281 and 218 and/or SEQ ID NOs: 321, 91 and 311)

(t) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05093 (SEQ ID NOs: 216, 217 and 241 or 280, 281 and 241 and/or SEQ ID NOs: 90, 91 and 311)

(u) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05094 (SEQ ID NOs: 216, 217 and 242 or 280, 281 and 242 and/or SEQ ID NOs: 90, 91 and 311)

(v) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05095 (SEQ ID NOs: 216, 217 and 243 or 280, 281 and 243 and/or SEQ ID NOs: 90, 91 and 311)

(w) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05096 (SEQ ID NOs: 216, 217 and 244 or 280, 281 and 244 and/or SEQ ID NOs: 90, 91 and 311)

(x) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05097 (SEQ ID NOs: 217, 216 and 245 or 280, 281 and 245 and/or SEQ ID NOs: 90, 91 and 311)

(y) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05098 (SEQ ID NOs: 219, 220 and 246 or 282, 283 and 246 and/or SEQ ID NOs: 312, 91 and 313)

(z) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05099 (SEQ ID NOs: 222, 223 and 224 or 288, 285 and 224 and/or SEQ ID NOs: 90, 91 and 311)

(aa) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05100 (SEQ ID NOs: 222, 223 and 247 or 288, 285 and 247 and/or SEQ ID NOs: 90, 91 and 311)

(ab) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab1 (SEQ ID NOs: 248, 249 and 250 or 289, 290 and 250 and/or SEQ ID NOs: 90, 91 and 322)

(ac) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab2 (SEQ ID NOs: 251, 252 and 253 or 291, 292 and 253 and/or SEQ ID NOs: 90, 91 and 323)

(ad) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab3 (SEQ ID NOs: 254, 255 and 256 or 293, 294 and 256 and/or SEQ ID NOs: 324, 325 and 326)

(ae) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab4 (SEQ ID NOs: 257, 258 and 259 or 295, 296 and 259 and/or SEQ ID NOs: 90, 91 and 327)

(af) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab5 (SEQ ID NOs: 260, 261 and 262 or 297, 298 and 262 and/or SEQ ID NOs: 324, 325 and 328)

(ag) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab6 (SEQ ID NOs: 263, 264 and 265 or 299, 300 and 265 and/or SEQ ID NOs: 324, 325 and 329)

(ah) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab7 (SEQ ID NOs: 266, 267 and 268 or 301, 302 and 268 and/or SEQ ID NOs: 90, 91 and 330)

(ai) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab8 (SEQ ID NOs: 269, 270 and 271 or 303, 304 and 271 and/or SEQ ID NOs: 90, 91 and 331)

(aj) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab9 (SEQ ID NOs: 272, 335 and 273 or 305, 306 and 273 and/or SEQ ID NOs: 90, 91 and 332)

(ak) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab10 (SEQ ID NOs: 274, 275 and 276 or 307, 308 and 276 and/or SEQ ID NOs: 90, 91 and 333) and/or

(al) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab11 (SEQ ID NOs: 277, 278 and 279 or 309, 310 and 279 and/or SEQ ID NOs: 324, 325 and 334).

Thus, binding domain B2 may comprise:

(a) the heavy chain variable region and/or the light chain variable region of antibody AC_05059 (SEQ ID NO: 33 and/or SEQ ID NO: 31)

(b) the heavy chain variable region and/or the light chain variable region of antibody AC_05060 (SEQ ID NO: 37 and/or SEQ ID NO: 35)

(c) the heavy chain variable region and/or the light chain variable region of antibody AC_05061 (SEQ ID NO: 41 and/or SEQ ID NO: 39)

(d) the heavy chain variable region and/or the light chain variable region of antibody AC_05062 (SEQ ID NO: 45 and/or SEQ ID NO: 43)

(e) the heavy chain variable region and/or the light chain variable region of antibody AC_05064 (SEQ ID NO: 49 and/or SEQ ID NO: 47)

(f) the heavy chain variable region and/or the light chain variable region of antibody AC_05079 (SEQ ID NO: 53 and/or SEQ ID NO: 51)

(g) the heavy chain variable region and/or the light chain variable region of antibody AC_05080 (SEQ ID NO: 57 and/or SEQ ID NO: 55)

(h) the heavy chain variable region and/or the light chain variable region of antibody AC_05081 (SEQ ID NO: 61 and/or SEQ ID NO: 59)

(i) the heavy chain variable region and/or the light chain variable region of antibody AC_05082 (SEQ ID NO: 65 and/or SEQ ID NO: 63)

(j) the heavy chain variable region and/or the light chain variable region of antibody AC_05083 (SEQ ID NO: 69 and/or SEQ ID NO: 67)

(k) the heavy chain variable region and/or the light chain variable region of antibody AC_05084 (SEQ ID NO: 106 and/or SEQ ID NO: 71)

(l) the heavy chain variable region and/or the light chain variable region of antibody AC_05085 (SEQ ID NO: 110 and/or SEQ ID NO: 108)

(m) the heavy chain variable region and/or the light chain variable region of antibody AC_05086 (SEQ ID NO: 114 and/or SEQ ID NO: 112)

(n) the heavy chain variable region and/or the light chain variable region of antibody AC_05087 (SEQ ID NO: 118 and/or SEQ ID NO: 116)

(o) the heavy chain variable region and/or the light chain variable region of antibody AC_05088 (SEQ ID NO: 122 and/or SEQ ID NO: 120)

(p) the heavy chain variable region and/or the light chain variable region of antibody AC_05089 (SEQ ID NO: 126 and/or SEQ ID NO: 124)

(q) the heavy chain variable region and/or the light chain variable region of antibody AC_05090 (SEQ ID NO: 130 and/or SEQ ID NO: 128)

(r) the heavy chain variable region and/or the light chain variable region of antibody AC_05091 (SEQ ID NO: 134 and/or SEQ ID NO: 132)

(s) the heavy chain variable region and/or the light chain variable region of antibody AC_05092 (SEQ ID NO: 138 and/or SEQ ID NO: 136)

(t) the heavy chain variable region and/or the light chain variable region of antibody AC_05093 (SEQ ID NO: 142 and/or SEQ ID NO: 140)

(u) the heavy chain variable region and/or the light chain variable region of antibody AC_05094 (SEQ ID NO: 146 and/or SEQ ID NO: 144)

(v) the heavy chain variable region and/or the light chain variable region of antibody AC_05095 (SEQ ID NO: 150 and/or SEQ ID NO: 148)

(w) the heavy chain variable region and/or the light chain variable region of antibody AC_05096 (SEQ ID NO: 154 and/or SEQ ID NO: 152)

(x) the heavy chain variable region and/or the light chain variable region of antibody AC_05097 (SEQ ID NO: 158 and/or SEQ ID NO: 156)

(y) the heavy chain variable region and/or the light chain variable region of antibody AC_05098 (SEQ ID NO: 162 and/or SEQ ID NO: 160)

(z) the heavy chain variable region and/or the light chain variable region of antibody AC_05099 (SEQ ID NO: 166 and/or SEQ ID NO: 164)

(aa) the heavy chain variable region and/or the light chain variable region of antibody AC_05100 (SEQ ID NO: 170 and/or SEQ ID NO: 168)

(ab) the heavy chain variable region and/or the light chain variable region of antibody Fab1 (SEQ ID NO: 174 and/or SEQ ID NO: 172)

(ac) the heavy chain variable region and/or the light chain variable region of antibody Fab2 (SEQ ID NO: 178 and/or SEQ ID NO: 176)

(ad) the heavy chain variable region and/or the light chain variable region of antibody Fab3 (SEQ ID NO: 182 and/or SEQ ID NO: 180)

(ae) the heavy chain variable region and/or the light chain variable region of antibody Fab4 (SEQ ID NO: 186 and/or SEQ ID NO: 184)

(af) the heavy chain variable region and/or the light chain variable region of antibody Fab5 (SEQ ID NO: 190 and/or SEQ ID NO: 188)

(ag) the heavy chain variable region and/or the light chain variable region of antibody Fab6 (SEQ ID NO: 194 and/or SEQ ID NO: 192)

(ah) the heavy chain variable region and/or the light chain variable region of antibody Fab7 (SEQ ID NO: 198 and/or SEQ ID NO: 196)

(ai) the heavy chain variable region and/or the light chain variable region of antibody Fab8 (SEQ ID NO: 202 and/or SEQ ID NO: 200)

(aj) the heavy chain variable region and/or the light chain variable region of antibody Fab9 (SEQ ID NO: 206 and/or SEQ ID NO: 204)

(ak) the heavy chain variable region and/or the light chain variable region of antibody Fab10 (SEQ ID NO: 210 and/or SEQ ID NO: 208)

(al) the heavy chain variable region and/or the light chain variable region of antibody Fab11 (SEQ ID NO: 214 and/or SEQ ID NO: 212)

(am) the heavy chain variable region and/or the light chain variable region of antibody mAb2 (SEQ ID NO: 387 and /or SEQ ID NO: 385) and/or

(an) the heavy chain variable region and/or the light chain variable region of antibody ffAC_05337 (SEQ ID NO: 433 and /or SEQ ID NO: 432).

In one embodiment, binding domain B2 may comprise:

(a) the light chain and/or the heavy chain of antibody AC_05059 (SEQ ID NO: 388 and/or SEQ ID NO: 389)

(b) the light chain and/or the heavy chain of antibody AC_05060 (SEQ ID NO: 390 and/or SEQ ID NO: 391)

(c) the light chain and/or the heavy chain of antibody AC_05061 (SEQ ID NO: 392 and/or (SEQ ID NO: 393)

(d) the light chain and/or the heavy chain of antibody AC_05062 (SEQ ID NO: 394 and/or SEQ ID NO: 395)

(e) the light chain and/or the heavy chain of antibody AC_05064 (SEQ ID NO: 396 and/or SEQ ID NO: 397)

(f) the light chain and/or the heavy chain of antibody AC_05079 (SEQ ID NO: 398 and/or SEQ ID NO: 399)

(g) the light chain and/or the heavy chain of antibody AC_05081 (SEQ ID NO: 400 and/or SEQ ID NO: 401)

(h) the light chain and/or the heavy chain of antibody AC_05088 (SEQ ID NO: 402 and/or SEQ ID NO: 403)

(i) the light chain and/or the heavy chain of antibody AC_05089 (SEQ ID NO: 404 and/or SEQ ID NO: 405)

(j) the light chain and/or the heavy chain of antibody AC_05090 (SEQ ID NO: 406 and/or SEQ ID NO: 407)

(k) the light chain and/or the heavy chain of antibody AC_05091 (SEQ ID NO: 408 and/or SEQ ID NO: 409)

(l) the light chain and/or the heavy chain of antibody AC_05093 (SEQ ID NO: 410 and/or SEQ ID NO: 411)

(m) the light chain and/or the heavy chain of antibody AC_05094 (SEQ ID NO: 412 and/or SEQ ID NO: 413)

(n) the light chain and/or the heavy chain of antibody AC_05096 (SEQ ID NO: 414 and/or SEQ ID NO: 415)

(o) the light chain and/or the heavy chain of antibody AC_05097 (SEQ ID NO: 416 and/or SEQ ID NO: 417)

(p) the light chain and/or the heavy chain of antibody Fab1 (SEQ ID NO: 418 and/or SEQ ID NO: 419) and/or

(q) the light chain and/or the heavy chain of antibody Fab3 (SEQ ID NO: 420 and/or (SEQ ID NO: 421).

It will be appreciated by the skilled person, and it is included herein, that mutations described herein for the RUBY™ format and/or the optimised RUBY™ format can be applied to the above light chain and/or the heavy chain sequences of the binding domain B2.

In one embodiment binding domain B2 may comprise one or more variants of the above-defined light chain variable regions and/or said heavy chain variable regions (and/or light chain and/or heavy chain) having at least 90% sequence identity thereto or 95% sequence identity thereto or 99% sequence identity thereto. Binding domain B2 may also comprise variants of the CDR sequences specified herein, for example variants where up one, two, three, four or five amino acid residues are substituted, deleted to added compared to the specified reference sequences.

In a second aspect, the present invention provides a polypeptide comprising a binding domain, designated B2, as defined herein, which is capable of specifically binding to CEA. Preferably, the polypeptide of the second aspect of the invention is monospecific. In an alternative preferred embodiment, the polypeptide of the second aspect of the invention is bispecific, preferably a bispecific antibody.

All of the features of B2 as discussed for the first aspect of the invention are relevant to, and included in, the second aspect of the invention. Accordingly, in one

embodiment the CEA binding domain of B2 is selected from: AC_05059; AC_05060; AC_05061; AC_05062; AC_05064; AC_05079; AC_05080; AC_05081; AC_05082; AC_05083; AC_05084; AC_05085; AC_05086; AC_05087; AC_05088; AC_05089; AC_05090; AC_05091; AC_05092; AC_05093; AC_05094; AC_05095; AC_05096; AC_05097; AC_05098; AC_05099; CEA binding domain of ffAC_05337 and AC_05100; Fab1; Fab2; Fab3; Fab4; Fab5; Fab6; Fab7; Fab8; Fab9; Fab10; Fab11; and mAb2, preferably: AC_05059; AC_05060; AC_05061; AC_05062; AC_05064; AC_05079; AC_05081; AC_05088; AC_05089; AC_05090; AC_05091; AC_05093; AC_05094; AC_05096; and AC_05097; Fab1; and Fab3, most preferably AC_05088; AC_05090; AC_05093; and AC_05097; Fab1; the CEA binding domain of ffAC_05337 and Fab3. The CDRs, VL, VH, light chain and/or heavy chain of the above antibodies as described in the first aspect of the invention are relevant to, and included in, the second aspect of the invention.

Advantageously, binding domain B2 of the second aspect of the invention binds to human CEA with a K_D of less than $2x10^{-6}$M or less than $1.5x10^{-7}$M or less than $1.5x10^{-8}$M or less than $2.5x10^{-8}$M or less than $4.5x10^{-8}$M or less than $5.5x10^{-8}$M or less than $6.5x10^{-9}$M or less than $2.5x10^{-9}$M or less than $2x10^{-9}$M or less than $9.5x10^{-10}$M or less than $4.5x10^{-10}$M or less than $7.5x10^{-11}$M or less than $8.5x10^{-12}$M or less than $1.5x10^{-12}$M or less than $1x10^{-12}$M, preferably less than $1.5x10^{-8}$M or less than $2.5x10^{-9}$M or less than $1.5x10^{-12}$M. Preferably, the K_D is measured in Octet; for example, as explained in the Examples. Preferably, the K_D is measured in Octet; for example, as explained in the Examples.

In a third aspect, the present invention provides a bispecific polypeptide comprising a first binding domain, designated B3, which is capable of binding specifically to a target antigen that is not CD40, and a second binding domain, designated B2, as defined herein, which is capable of specifically binding to CEA.

All of the features of B2 as discussed for the first and second aspect of the invention are relevant to, and included in, the third aspect of the invention. Accordingly, in one embodiment the CEA binding domain of B2 is selected from: AC_05059; AC_05060; AC_05061; AC_05062; AC_05064; AC_05079; AC_05080; AC_05081; AC_05082; AC_05083; AC_05084; AC_05085; AC_05086; AC_05087; AC_05088; AC_05089; AC_05090; AC_05091; AC_05092; AC_05093; AC_05094; AC_05095; AC_05096; AC_05097; AC_05098; AC_05099; and AC_05100; Fab1; Fab2; Fab3; Fab4; Fab5; Fab6; Fab7; Fab8; Fab9; Fab10; Fab11; CEA binding domain of ffAC_05337 and mAb2, preferably: AC_05059; AC_05060; AC_05061; AC_05062; AC_05064; AC_05079;

AC_05081; AC_05088; AC_05089; AC_05090; AC_05091; AC_05093; AC_05094; AC_05096; and AC_05097; Fab1; and Fab3, most preferably AC_05088; AC_05090; AC_05093; and AC_05097; Fab1; CEA binding domain of ffAC_05337 and Fab3. The CDRs, VL, VH, light chain and/or heavy chain of the above antibodies as described in the first aspect of the invention are relevant to, and included in, the second aspect of the invention.

Preferably, the target antigen is protein and/or a peptide.

In one embodiment, B3 is capable of targeting an immune cell, such that the target antigen is an immune cell target antigen. The immune cell may be any immune cell described herein, including a T cell, a NK cell, a myeloid cell, and/or an antigen presenting cell (such as a dendritic cell).

In one embodiment, B3 is capable of targeting a dendritic cell (DC), such that the target antigen is a DC target antigen.

Binding domain B3 specifically binds to the DC target antigen, i.e. it binds to the DC target antigen but does not bind, or binds at a lower affinity, to other molecules. Binding domain B3 may have some binding affinity for the same DC target antigen from other mammals, such as from a non-human primate (for example *Macaca fascicularis* (cynomolgus monkey), *Macaca mulatta*). Binding domain B3 preferably does not bind non-target molecules.

Binding domain B3 is preferably capable of binding to its target with an affinity that is at least two-fold, 10-fold, 50-fold, 100-fold or greater than its affinity for binding to another non-target molecule.

In one embodiment, the DC target antigen is capable of mediating internalisation.

In one embodiment, the DC target antigen is capable of mediating cross-presentation.

In one embodiment, the DC target antigen is specifically expressed on mature DCs.

In one embodiment, the DC target antigen is specifically expressed on immature DCs.

In a preferred embodiment of the third aspect of the invention, the bispecific polypeptide is a bispecific antibody, such as a bispecific antibody in the RUBY™ format or optimised RUBY™ format, as both described herein.

Exemplary CD40–CEA bispecific antibodies

In one embodiment of the bispecific polypeptides of the invention, binding domain B1 is an IgG and binding domain B2 is an scFv. Conversely, binding domain B1 may be an scFv and binding domain B2 may be an IgG.

In one embodiment binding domain B1 is an immunoglobulin and binding domain B2 is a Fab. Conversely, binding domain B1 may be a Fab and binding domain B2 may be an immunoglobulin. The bispecific polypeptide may optionally be in the RUBY™ format or optimised RUBY™ format, as both described herein. The bispecific polypeptide format is as described above and as laid out in Figure 23, and the bispecific polypeptide may comprise certain mutations as described above.

Bispecific polypeptides of the invention may comprise the CDRs of the light chains of any of the B1 domains described above (as laid out in Table C(2) below), and/or the CDRs of the heavy chains of any of the B1 domains described above (as laid out in Table C(1) below), in combination with any of the CDRs of the light chains of any of the B2 domains described above (as laid out in Table D(2)), and/or the CDRs of the heavy chains of any of the B2 domains described above (as laid out in Table D(1a) and/or Table D(1b)).

Bispecific polypeptides of the invention may comprise the light chain variable regions of any of the B1 domains described above (as laid out in Table A below), and/or the CDRs of the heavy chain variable regions of any of the B1 domains described above (as laid out in Table A below), in combination with any of the light chain variable regions of any of the B2 domains described above (as laid out in Table B), and/or the heavy chain variable regions of any of the B2 domains described above (as laid out in Table B).

Thus, in certain embodiments B1 and B2 comprise the respective variable regions comprising the CDRs identified above. For example, B1 may comprise the heavy chain variable region and/or the light chain variable region of antibody G12 (SEQ ID NO: 19 and/or SEQ ID NO: 17) or G12_mut (SEQ ID NO: 29 and/or SEQ ID NO: 17) and B2

may comprise the heavy chain variable region and/or the light chain variable region of any of the reference CEA antibodies:

(a) the heavy chain variable region and/or the light chain variable region of antibody AC_05059 (SEQ ID NO: 33 and/or SEQ ID NO: 31)

(b) the heavy chain variable region and/or the light chain variable region of antibody AC_05060 (SEQ ID NO: 37 and/or SEQ ID NO: 35)

(c) the heavy chain variable region and/or the light chain variable region of antibody AC_05061 (SEQ ID NO: 41 and/or SEQ ID NO: 39)

(d) the heavy chain variable region and/or the light chain variable region of antibody AC_05062 (SEQ ID NO: 45 and/or SEQ ID NO: 43)

(e) the heavy chain variable region and/or the light chain variable region of antibody AC_05064 (SEQ ID NO: 49 and/or SEQ ID NO: 47)

(f) the heavy chain variable region and/or the light chain variable region of antibody AC_05079 (SEQ ID NO: 53 and/or SEQ ID NO: 51)

(g) the heavy chain variable region and/or the light chain variable region of antibody AC_05080 (SEQ ID NO: 57 and/or SEQ ID NO: 55)

(h) the heavy chain variable region and/or the light chain variable region of antibody AC_05081 (SEQ ID NO: 61 and/or SEQ ID NO: 59)

(i) the heavy chain variable region and/or the light chain variable region of antibody AC_05082 (SEQ ID NO: 65 and/or SEQ ID NO: 63)

(j) the heavy chain variable region and/or the light chain variable region of antibody AC_05083 (SEQ ID NO: 69 and/or SEQ ID NO: 67)

(k) the heavy chain variable region and/or the light chain variable region of antibody AC_05084 (SEQ ID NO: 106 and/or SEQ ID NO: 71)

(l) the heavy chain variable region and/or the light chain variable region of antibody AC_05085 (SEQ ID NO: 110 and/or SEQ ID NO: 108)

(m) the heavy chain variable region and/or the light chain variable region of antibody AC_05086 (SEQ ID NO: 114 and/or SEQ ID NO: 112)

(n) the heavy chain variable region and/or the light chain variable region of antibody AC_05087 (SEQ ID NO: 118 and/or SEQ ID NO: 116)

(o) the heavy chain variable region and/or the light chain variable region of antibody AC_05088 (SEQ ID NO: 122 and/or SEQ ID NO: 120)

(p) the heavy chain variable region and/or the light chain variable region of antibody AC_05089 (SEQ ID NO: 126 and/or SEQ ID NO: 124)

(q) the heavy chain variable region and/or the light chain variable region of antibody AC_05090 (SEQ ID NO: 130 and/or SEQ ID NO: 128)

(r) the heavy chain variable region and/or the light chain variable region of antibody AC_05091 (SEQ ID NO: 134 and/or SEQ ID NO: 132)

(s) the heavy chain variable region and/or the light chain variable region of antibody AC_05092 (SEQ ID NO: 138 and/or SEQ ID NO: 136)

(t) the heavy chain variable region and/or the light chain variable region of antibody AC_05093 (SEQ ID NO: 142 and/or SEQ ID NO: 140)

(u) the heavy chain variable region and/or the light chain variable region of antibody AC_05094 (SEQ ID NO: 146 and/or SEQ ID NO: 144)

(v) the heavy chain variable region and/or the light chain variable region of antibody AC_05095 (SEQ ID NO: 150 and/or SEQ ID NO: 148)

(w) the heavy chain variable region and/or the light chain variable region of antibody AC_05096 (SEQ ID NO: 154 and/or SEQ ID NO: 152)

(x) the heavy chain variable region and/or the light chain variable region of antibody AC_05097 (SEQ ID NO: 158 and/or SEQ ID NO: 156)

(y) the heavy chain variable region and/or the light chain variable region of antibody AC_05098 (SEQ ID NO: 162 and/or SEQ ID NO: 160)

(z) the heavy chain variable region and/or the light chain variable region of antibody AC_05099 (SEQ ID NO: 166 and/or SEQ ID NO: 164)

(aa) the heavy chain variable region and/or the light chain variable region of antibody AC_05100 (SEQ ID NO: 170 and/or SEQ ID NO: 168)

(ab) the heavy chain variable region and/or the light chain variable region of antibody Fab1 (SEQ ID NO: 174 and/or SEQ ID NO: 172)

(ac) the heavy chain variable region and/or the light chain variable region of antibody Fab2 (SEQ ID NO: 178 and/or SEQ ID NO: 176)

(ad) the heavy chain variable region and/or the light chain variable region of antibody Fab3 (SEQ ID NO: 182 and/or SEQ ID NO: 180)

(ae) the heavy chain variable region and/or the light chain variable region of antibody Fab4 (SEQ ID NO: 186 and/or SEQ ID NO: 184)

(af) the heavy chain variable region and/or the light chain variable region of antibody Fab5 (SEQ ID NO: 190 and/or SEQ ID NO: 188)

(ag) the heavy chain variable region and/or the light chain variable region of antibody Fab6 (SEQ ID NO: 194 and/or SEQ ID NO: 192)

(ah) the heavy chain variable region and/or the light chain variable region of antibody Fab7 (SEQ ID NO: 198 and/or SEQ ID NO: 196)

(ai) the heavy chain variable region and/or the light chain variable region of antibody Fab8 (SEQ ID NO: 202 and/or SEQ ID NO: 200)

(aj) the heavy chain variable region and/or the light chain variable region of antibody Fab9 (SEQ ID NO: 206 and/or SEQ ID NO: 204)

(ak) the heavy chain variable region and/or the light chain variable region of antibody Fab10 (SEQ ID NO: 210 and/or SEQ ID NO: 208)

(al) the heavy chain variable region and/or the light chain variable region of antibody Fab11 (SEQ ID NO: 214 and/or SEQ ID NO: 212) and/or
(am) the heavy chain variable region and/or the light chain variable region of antibody mAb2 (SEQ ID NO: 387 and /or SEQ ID NO: 385).

In a preferred embodiment, B1 may comprise the heavy chain variable region and/or the light chain variable region of antibody ffAC_05337 (SEQ ID NO: 430 and/or SEQ ID NO: 431) and B2 may comprise the heavy chain variable region and/or the light chain variable region of antibody ffAC_05337 (SEQ ID NO: 433 and/or SEQ ID NO: 432).

The B1 domain may comprise the light chain variable region and/or the heavy chain variable region of any B1 domain described above, and the B2 domain may comprise the light chain variable region and/or the heavy chain variable region of any B2 domain described above, or variants of said light chain variable regions and/or said heavy chain variable regions having at least 90% sequence identity thereto.

Typically, the bispecific polypeptides of the invention will comprise constant region sequences, in addition to the above-defined variable region sequences. Bispecific polypeptides of the invention may be in any suitable format. For example, bispecific polypeptides may be in the RUBY™ format or optimised RUBY™ format (as described above, and shown in Figure 23), or in the Morrison format.

An exemplary heavy chain constant region amino acid sequence which may be combined with any VH region sequence disclosed herein (to form a complete heavy chain) is the following IgG1 heavy chain constant region sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK
(SEQ ID NO: 349)

Likewise, an exemplary light chain constant region amino acid sequence which may be combined with any VL region sequence disclosed herein (to form a complete light chain) is the Kappa chain constant region sequence reproduced here:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
(SEQ ID NO: 350)

Other light chain constant region sequences are known in the art and could also be combined with any VL region disclosed herein.

In one embodiment, the polypeptide may comprise the following constant region amino acid sequences:

(a) Reference sequence CH1 (SEQ ID NO: 354):
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV**EPKSC**
(wherein the bold and underlined section is part of the hinge region, but is present in the Fab fragment)

and/or

(b) Reference sequence CKappa (SEQ ID NO: 355):
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

and/or

Reference sequence CLambda (SEQ ID NO: 356)
GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNN
KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

and/or

Reference sequence CLambda (SEQ ID NO: 357)
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN
KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

and/or

Reference sequence CLambda (SEQ ID NO: 358)

GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN
KYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS

As described above, these reference sequences may comprise one or more mutations to prevent the formation of aggregates and/or a Fab by-product. Such mutation positions (identified earlier in the description) may be given relative to any of the above constant region sequences.

In one embodiment, the bispecific polypeptide is in the RUBY™ format or in the optimised RUBY™ format, comprising an immunoglobulin and a Fab fragment, wherein the Fab fragment is fused to the C-terminus of the heavy chain of the immunoglobulin via the light chain of the Fab fragment.

Thus in one embodiment, binding domain B1 is an immunoglobulin, and binding domain B2 is a Fab fragment, and the Fab fragment is fused to the C-terminus of the heavy chain of the immunoglobulin via the light chain of the Fab fragment. In an alternative embodiment, binding domain B2 is an immunoglobulin, and binding domain B1 is a Fab fragment, and the Fab fragment is fused to the C-terminus of the heavy chain of the immunoglobulin via the light chain of the Fab fragment. Additionally, the bispecific polypeptide comprises one or more mutations selected from those described above for the RUBY™ format and/or the optimised RUBY™ format.

In one embodiment, the bispecific polypeptide comprises a binding domain B1 and/or a binding domain 2 comprising the light chain CDRs and/or heavy chain CDRs, and/or the format, of an antibody selected from the list consisting of: Multi1; Multi2; Multi3; Multi4; Multi5; Multi6; Multi7; Multi8; Multi9; Multi10; Multi11; Multi12; Multi13; Multi14; Multi17; Multi18; Multi19; Multi20; Multi23; Multi24; Multi25; Multi26; Multi27; Multi28; Multi29; Multi30; Multi31; Multi32; Multi33; Multi34; Multi35; Multi37; Multi38; Multi39; Multi40; Multi41; Multi42; Multi44; Multi46; Multi47; Multi48; Multi49; AC_05333; AC_05334; AC_05336; AC_05337; AC_05338; AC_05339; AC_05341; ffAC_05337; ffAC_05339; and/or AC_05355, preferably Multi34; Multi42; Multi46 and/or ffAC_05337.

In one embodiment, the bispecific polypeptide comprises a binding domain B1 and/or a binding domain 2 comprising the light chain variable region and/or heavy chain variable region, and/or the format, of an antibody selected from the list consisting of: Multi1; Multi2; Multi3; Multi4; Multi5; Multi6; Multi7; Multi8; Multi9; Multi10; Multi11; Multi12; Multi13; Multi14; Multi17; Multi18; Multi19; Multi20; Multi23; Multi24;

Multi25; Multi26; Multi27; Multi28; Multi29; Multi30; Multi31; Multi32; Multi33; Multi34; Multi35; Multi37; Multi38; Multi39; Multi40; Multi41; Multi42; Multi44; Multi46; Multi47; Multi48; Multi49; AC_05333; AC_05334; AC_05336; AC_05337; AC_05338; AC_05339; AC_05341; ffAC_05337; ffAC_05339; and/or AC_05355, preferably Multi34; Multi42; Multi46 and/or ffAC_05337.

In one embodiment, the bispecific polypeptide comprises a binding domain B1 and/or a binding domain 2 comprising the light chain and/or heavy chain, and/or the format, of an antibody selected from the list consisting of: Multi1; Multi2; Multi3; Multi4; Multi5; Multi6; Multi7; Multi8; Multi9; Multi10; Multi11; Multi12; Multi13; Multi14; Multi17; Multi18; Multi19; Multi20; Multi23; Multi24; Multi25; Multi26; Multi27; Multi28; Multi29; Multi30; Multi31; Multi32; Multi33; Multi34; Multi35; Multi37; Multi38; Multi39; Multi40; Multi41; Multi42; Multi44; Multi46; Multi47; Multi48; Multi49; AC_05333; AC_05334; AC_05336; AC_05337; AC_05338; AC_05339; AC_05341; ffAC_05337; ffAC_05339; and/or AC_05355, preferably Multi34; Multi42; Multi46 and/or ffAC_05337.

In one embodiment, the bispecific polypeptide comprises a Chain H1 comprising a sequence selected from the listing consisting of: SEQ ID NO: 359; SEQ ID NO: 362; SEQ ID NO: 365; and/or SEQ ID NO: 367.

In one embodiment, the bispecific polypeptide comprises a Chain L1 comprising a sequence selected from the listing consisting of: SEQ ID NO: 360; SEQ ID NO: 363; SEQ ID NO: 372; and/or SEQ ID NO: 368.

In one embodiment, the bispecific polypeptide comprises a Chain H2 comprising a sequence selected from the listing consisting of: SEQ ID NO: 361; SEQ ID NO: 364; SEQ ID NO: 366; and/or SEQ ID NO: 369.

In one embodiment, the bispecific polypeptide:

- comprises a Chain H1 comprising a sequence selected from the listing consisting of: SEQ ID NO: 359; SEQ ID NO: 362; SEQ ID NO: 365; and/or SEQ ID NO: 367; and/or
- comprises a Chain L1 comprising a sequence selected from the listing consisting of: SEQ ID NO: 360; SEQ ID NO: 363; SEQ ID NO: 372; and/or SEQ ID NO: 368; and/or

- comprises a Chain H2 comprising a sequence selected from the listing consisting of: SEQ ID NO: 361; SEQ ID NO: 364; SEQ ID NO: 366; and/or SEQ ID NO: 369.

In one embodiment, the bispecific polypeptide:

- comprises two Chain H1 each comprising a sequence selected from the listing consisting of: SEQ ID NO: 359; SEQ ID NO: 362; SEQ ID NO: 365; and/or SEQ ID NO: 367; and/or
- comprises two Chain L1 each comprising a sequence selected from the listing consisting of: SEQ ID NO: 360; SEQ ID NO: 363; SEQ ID NO: 372; and/or SEQ ID NO: 368; and/or
- comprises two Chain H2 each comprising a sequence selected from the listing consisting of: SEQ ID NO: 361; SEQ ID NO: 364; SEQ ID NO: 366; and/or SEQ ID NO: 369.

In one embodiment the bispecific polypeptide may comprise one or more variants of the above-defined Chain H1, Chain L1, and/or Chain H2 having at least 90% sequence identity thereto or 95% sequence identity thereto or 99% sequence identity thereto.

In one embodiment, the bispecific polypeptide:

- comprises a Chain H1 comprising SEQ ID NO: 359; and/or
- comprises a Chain L1 comprising SEQ ID NO: 360; and/or
- comprises a Chain H2 comprising SEQ ID NO: 361.

In one embodiment, the bispecific polypeptide:

- comprises a Chain H1 comprising SEQ ID NO: 362; and/or
- comprises a Chain L1 comprising SEQ ID NO: 363; and/or
- comprises a Chain H2 comprising SEQ ID NO: 364;.

In one embodiment, the bispecific polypeptide:

- comprises a Chain H1 comprising SEQ ID NO: 365; and/or
- comprises a Chain L1 comprising SEQ ID NO: 372; and/or
- comprises a Chain H2 comprising SEQ ID NO: 366.

In one embodiment, the bispecific polypeptide:

- comprises a Chain H1 comprising SEQ ID NO: 367; and/or
- comprises a Chain L1 comprising SEQ ID NO: 368; and/or
- comprises a Chain H2 comprising SEQ ID NO: 369.

In one embodiment, the bispecific polypeptide:

- comprises two Chain H1 comprising SEQ ID NO: 359; and/or
- comprises two Chain L1 comprising SEQ ID NO: 360; and/or
- comprises two Chain H2 comprising SEQ ID NO: 361.

In one embodiment, the bispecific polypeptide:

- comprises two Chain H1 comprising SEQ ID NO: 362; and/or
- comprises two Chain L1 comprising SEQ ID NO: 363; and/or
- comprises two Chain H2 comprising SEQ ID NO: 364;.

In one embodiment, the bispecific polypeptide:

- comprises two Chain H1 comprising SEQ ID NO: 365; and/or
- comprises two Chain L1 comprising SEQ ID NO: 372; and/or
- comprises two Chain H2 comprising SEQ ID NO: 366.

In one embodiment, the bispecific polypeptide:

- comprises two Chain H1 comprising SEQ ID NO: 367; and/or
- comprises two Chain L1 comprising SEQ ID NO: 368; and/or
- comprises two Chain H2 comprising SEQ ID NO: 369.

In one embodiment, the bispecific polypeptide is an antibody selected from the list consisting of: Multi1; Multi2; Multi3; Multi4; Multi5; Multi6; Multi7; Multi8; Multi9; Multi10; Multi11; Multi12; Multi13; Multi14; Multi17; Multi18; Multi19; Multi20; Multi23; Multi24; Multi25; Multi26; Multi27; Multi28; Multi29; Multi30; Multi31; Multi32; Multi33; Multi34; Multi35; Multi37; Multi38; Multi39; Multi40; Multi41; Multi42; Multi44; Multi46; Multi47; Multi48; Multi49; AC_05333; AC_05334; AC_05336; AC_05337; AC_05338; AC_05339; AC_05341; ffAC_05337; ffAC_05339; and/or AC_05355, preferably Multi34; Multi42; Multi46 and/or ffAC_05337.

In one embodiment the bispecific polypeptide may comprise one or more variants of the above-defined Multi1; Multi2; Multi3; Multi4; Multi5; Multi6; Multi7; Multi8; Multi9; Multi10; Multi11; Multi12; Multi13; Multi14; Multi17; Multi18; Multi19; Multi20; Multi23; Multi24; Multi25; Multi26; Multi27; Multi28; Multi29; Multi30; Multi31; Multi32; Multi33; Multi34; Multi35; Multi37; Multi38; Multi39; Multi40; Multi41; Multi42; Multi44; Multi46; Multi47; Multi48; Multi49; AC_05333; AC_05334; AC_05336; AC_05337; AC_05338; AC_05339; AC_05341; ffAC_05337; ffAC_05339;

and/or AC_05355 having at least 90% sequence identity thereto or 95% sequence identity thereto or 99% sequence identity thereto.

Exemplary full heavy and light chain sequences

Binding domain B1:

1132/1133 Heavy chain, including RUBY mutations (VH: Q44R, CH1: H168A, F170G, CH2: L234A, L235A) (SEQ ID NO: 371):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRRAPGKGLEWVSGIGSYGGGTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYVNFGMDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVATGPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

1132/1133 Light chain, including RUBY mutations (VL: Q44E, CKappa: L135Y, S176W) (SEQ ID NO: 372):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQEKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYGRNPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCYLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLWSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

1132/1133 Heavy chain (SEQ ID NO: 378):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGIGSYGGGTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYVNFGMDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

1132/1133 Light chain (SEQ ID NO: 379):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYGRNPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA

SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

G12 Heavy chain (SEQ ID NO: 380):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWLSYISGGSSYIFYADS
VRGRFTISRDNSENALYLQMNSLRAEDTAVYYCARILRGGSGMDLWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

G12 Light chain (SEQ ID NO: 381):
QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVYWYQQLPGTAPKLLIYGNINRPSGVPDRF
SGSKSGTSASLAISGLRSEDEADYYCAAWDKSISGLVFGGGTKLTVLGQPKAAPSVTLFPPSSE
ELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKS
HRSYSCQVTHEGSTVEKTVAPTECS

G12_mut Heavy chain (SEQ ID NO: 382):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWLSYISGGSSYIFYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARILRGGSGMDLWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

G12_mut Light chain (SEQ ID NO: 383):
QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVYWYQQLPGTAPKLLIYGNINRPSGVPDRF
SGSKSGTSASLAISGLRSEDEADYYCAAWDKSISGLVFGGGTKLTVLGQPKAAPSVTLFPPSSE
ELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKS
HRSYSCQVTHEGSTVEKTVAPTECS

Binding domain B2:

AC_05059, light chain (SEQ ID NO: 388):

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05059, heavy chain (SEQ ID NO: 389):EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYST
SYADSVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPHLDYWGQGTLVTVSSA
STKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKP
KDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

AC_05060, light chain (SEQ ID NO: 390):
DIQMTQSPSSLSASVGDRVTITCRASQSIRDYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQGTFPFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

AC_05060, heavy chain (SEQ ID NO: 391):
EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYYMSWVRQAPGKGLEWVSGISGYGYYTGYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHGYGVIDYWGQGTLVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT
PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

AC_05061, light chain (SEQ ID NO: 392):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQGAYVPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05061, heavy chain (SEQ ID NO: 393):

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYGYTHFDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

AC_05062, light chain (SEQ ID NO: 394):
DIQMTQSPSSLSASVGDRVTITCRASQAISGYLNWYQQKPGKAPKLLIYSASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQSYSTPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05062, heavy chain (SEQ ID NO: 395):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYRWHGSVFDYWGQGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05064, light chain (SEQ ID NO: 396):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYPWYFPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

AC_05064, heavy chain (SEQ ID NO: 397):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYGYSVLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES

NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

AC_05079, light chain (SEQ ID NO: 398):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05079, heavy chain (SEQ ID NO: 399):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPPLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05081, light chain (SEQ ID NO: 400):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05081, heavy chain (SEQ ID NO: 401):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFQPHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05088, light chain (SEQ ID NO: 402):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA

SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05088, heavy chain (SEQ ID NO: 403):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVLFPPHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05089, light chain (SEQ ID NO: 404):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05089, heavy chain (SEQ ID NO: 405):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPHHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05090, light chain (SEQ ID NO: 406):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05090, heavy chain (SEQ ID NO: 407):

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPLHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05091, light chain (SEQ ID NO: 408):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05091, heavy chain (SEQ ID NO: 409):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPHFDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05093, light chain (SEQ ID NO: 410):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05093, heavy chain (SEQ ID NO: 411):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPHVDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE

WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05094, light chain (SEQ ID NO: 412):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05094, heavy chain (SEQ ID NO: 413):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPHWDYWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

AC_05096, light chain (SEQ ID NO: 414):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05096, heavy chain (SEQ ID NO: 415):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFRPHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05097, light chain (SEQ ID NO: 416):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA

SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05097, heavy chain (SEQ ID NO: 417):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFSPHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

Fab1, light chain (SEQ ID NO: 418):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQSSHGPLLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

Fab1, heavy chain (SEQ ID NO: 419):
QVQLVQSGAEVKKPGSSVKVSCKASGGTFGYYAIHWVRQAPGQGLEWMGGIGSIFGTANYAQ
KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAWSSDHMDYWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

Fab3, light chain (SEQ ID NO: 420):
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDFAVYYCQQYWYPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

Fab3, heavy chain (SEQ ID NO: 421):
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSSIHWVRQAPGQGLEWMGHIYPSFGTANYAQ
KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHSGSRFFSPMDYWGQGTLVTVSSASTK

GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

As discussed above, methods for the production of polypeptides of the invention are well known in the art.

Conveniently, the polypeptide is or comprises a recombinant polypeptide. Suitable methods for the production of such recombinant polypeptides are well known in the art, such as expression in prokaryotic or eukaryotic hosts cells (for example, see Green & Sambrook, 2012, *Molecular Cloning, A Laboratory Manual*, Fourth Edition, Cold Spring Harbor, New York, the relevant disclosures in which document are hereby incorporated by reference).

Polypeptides of the invention can also be produced using a commercially available *in vitro* translation system, such as rabbit reticulocyte lysate or wheatgerm lysate (available from Promega). Preferably, the translation system is rabbit reticulocyte lysate. Conveniently, the translation system may be coupled to a transcription system, such as the TNT transcription-translation system (Promega). This system has the advantage of producing suitable mRNA transcript from an encoding DNA polynucleotide in the same reaction as the translation.

It will be appreciated by persons skilled in the art that polypeptides of the invention may alternatively be synthesised artificially, for example using well known liquid-phase or solid phase synthesis techniques (such as *t*-Boc or Fmoc solid-phase peptide synthesis).

Polynucleotides, vectors and cells

A fourth aspect of the invention provides an isolated nucleic acid molecule encoding a bispecific polypeptide according to any one of the preceding claims, or a component polypeptide chain thereof. For example, the nucleic acid molecule may comprise any of the nucleotide sequences provided in Tables A and B.

Thus, a polynucleotide of the invention may encode any polypeptide as described herein, or all or part of B1 or all or part of B2. The terms "nucleic acid molecule" and

"polynucleotide" are used interchangeably herein and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides, or analogs thereof. Non-limiting examples of polynucleotides include a gene, a gene fragment, messenger RNA (mRNA), cDNA, recombinant polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes, and primers. A polynucleotide of the invention may be provided in isolated or substantially isolated form. By substantially isolated, it is meant that there may be substantial, but not total, isolation of the polypeptide from any surrounding medium. The polynucleotides may be mixed with carriers or diluents which will not interfere with their intended use and still be regarded as substantially isolated.

A nucleic acid sequence which "encodes" a selected polypeptide is a nucleic acid molecule which is transcribed (in the case of DNA) and translated (in the case of mRNA) into a polypeptide in vivo when placed under the control of appropriate regulatory sequences. The boundaries of the coding sequence are determined by a start codon at the 5' (amino) terminus and a translation stop codon at the 3' (carboxy) terminus. For the purposes of the invention, such nucleic acid sequences can include, but are not limited to, cDNA from viral, prokaryotic or eukaryotic mRNA, genomic sequences from viral or prokaryotic DNA or RNA, and even synthetic DNA sequences. A transcription termination sequence may be located 3' to the coding sequence.

Representative polynucleotides which encode examples of a heavy chain or light chain amino acid sequence of an antibody may comprise or consist of any one of the nucleotide sequences disclosed herein, for example the sequences set out in Tables A and B.

A suitable polynucleotide sequence may alternatively be a variant of one of these specific polynucleotide sequences. For example, a variant may be a substitution, deletion or addition variant of any of the above nucleic acid sequences. A variant polynucleotide may comprise 1, 2, 3, 4, 5, up to 10, up to 20, up to 30, up to 40, up to 50, up to 75 or more nucleic acid substitutions and/or deletions from the sequences given in the sequence listing.

Suitable variants may be at least 70% homologous to a polynucleotide of any one of nucleic acid sequences disclosed herein, preferably at least 80 or 90% and more preferably at least 95%, 97% or 99% homologous thereto. Preferably homology and identity at these levels is present at least with respect to the coding regions of the polynucleotides. Methods of measuring homology are well known in the art and it will

be understood by those of skill in the art that in the present context, homology is calculated on the basis of nucleic acid identity. Such homology may exist over a region of at least 15, preferably at least 30, for instance at least 40, 60, 100, 200 or more contiguous nucleotides. Such homology may exist over the entire length of the unmodified polynucleotide sequence.

Methods of measuring polynucleotide homology or identity are known in the art. For example the UWGCG Package provides the BESTFIT program which can be used to calculate homology (e.g. used on its default settings) (Devereux et al, 1984; the disclosures of which are incorporated herein by reference).

The PILEUP and BLAST algorithms can also be used to calculate homology or line up sequences (typically on their default settings), for example as described in Altschul, 1993; Altschul et al, 1990, the disclosures of which are incorporated herein by reference).

Software for performing BLAST analysis is publicly available through the National Centre for Biotechnology Information (http://www.ncbi.nlm.nih.gov/). This algorithm involves first identifying high scoring sequence pair (HSPs) by identifying short words of length W in the query sequence that either match or satisfy some positive-valued threshold score T when aligned with a word of the same length in a database sequence. T is referred to as the neighbourhood word score threshold (Altschul et al, supra). These initial neighbourhood word hits act as seeds for initiating searches to find HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Extensions for the word hits in each direction are halted when: the cumulative alignment score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters W, T and X determine the sensitivity and speed of the alignment. The BLAST program uses as defaults a word length (W) of 11, the BLOSUM62 scoring matrix (see Henikoff & Henikoff, 1992; the disclosures of which are incorporated herein by reference) alignments (B) of 50, expectation (E) of 10, M=5, N=4, and a comparison of both strands.

The BLAST algorithm performs a statistical analysis of the similarity between two sequences; see e.g. Karlin & Altschul, 1993; the disclosures of which are incorporated herein by reference. One measure of similarity provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an indication of the probability by

which a match between two nucleotide or amino acid sequences would occur by chance. For example, a sequence is considered similar to another sequence if the smallest sum probability in comparison of the first sequence to the second sequence is less than about 1, preferably less than about 0.1, more preferably less than about 0.01, and most preferably less than about 0.001.

The homologue may differ from a sequence in the relevant polynucleotide by less than 3, 5, 10, 15, 20 or more mutations (each of which may be a substitution, deletion or insertion). These mutations may be measured over a region of at least 30, for instance at least 40, 60 or 100 or more contiguous nucleotides of the homologue.

In one embodiment, a variant sequence may vary from the specific sequences given in the sequence listing by virtue of the redundancy in the genetic code. The DNA code has 4 primary nucleic acid residues (A, T, C and G) and uses these to "spell" three letter codons which represent the amino acids the proteins encoded in an organism's genes. The linear sequence of codons along the DNA molecule is translated into the linear sequence of amino acids in the protein(s) encoded by those genes. The code is highly degenerate, with 61 codons coding for the 20 natural amino acids and 3 codons representing "stop" signals. Thus, most amino acids are coded for by more than one codon - in fact several are coded for by four or more different codons. A variant polynucleotide of the invention may therefore encode the same polypeptide sequence as another polynucleotide of the invention, but may have a different nucleic acid sequence due to the use of different codons to encode the same amino acids.

A polypeptide of the invention may thus be produced from or delivered in the form of a polynucleotide which encodes, and is capable of expressing, it.

Polynucleotides of the invention can be synthesised according to methods well known in the art, as described by way of example in Green & Sambrook (2012, Molecular Cloning - a laboratory manual, 4th edition; Cold Spring Harbor Press; the disclosures of which are incorporated herein by reference).

The nucleic acid molecules of the present invention may be provided in the form of an expression cassette which includes control sequences operably linked to the inserted sequence, thus allowing for expression of the polypeptide of the invention in vivo. These expression cassettes, in turn, are typically provided within vectors (e.g., plasmids or recombinant viral vectors). Such an expression cassette may be administered directly to a host subject. Alternatively, a vector comprising a

polynucleotide of the invention may be administered to a host subject. Preferably the polynucleotide is prepared and/or administered using a genetic vector. A suitable vector may be any vector which is capable of carrying a sufficient amount of genetic information, and allowing expression of a polypeptide of the invention.

The present invention thus includes expression vectors that comprise such polynucleotide sequences. Such expression vectors are routinely constructed in the art of molecular biology and may for example involve the use of plasmid DNA and appropriate initiators, promoters, enhancers and other elements, such as for example polyadenylation signals which may be necessary, and which are positioned in the correct orientation, in order to allow for expression of a peptide of the invention. Other suitable vectors would be apparent to persons skilled in the art (see Green & Sambrook, *supra*).

The invention also includes cells that have been modified to express a polypeptide of the invention. Such cells include transient, or preferably stable higher eukaryotic cell lines, such as mammalian cells or insect cells, lower eukaryotic cells, such as yeast or prokaryotic cells such as bacterial cells. Particular examples of cells which may be modified by insertion of vectors or expression cassettes encoding for a polypeptide of the invention include mammalian human embryonic kidney (HEK) (for example, HEK293T), CHO, HeLa, NS0 and COS cells. Preferably the cell line selected will be one which is not only stable, but also allows for mature glycosylation and cell surface expression of a polypeptide.

Such cell lines of the invention may be cultured using routine methods to produce a polypeptide of the invention, or may be used therapeutically or prophylactically to deliver antibodies of the invention to a subject. Alternatively, polynucleotides, expression cassettes or vectors of the invention may be administered to a cell from a subject ex vivo and the cell then returned to the body of the subject.

In one embodiment, the nucleic acid molecule encodes an antibody heavy chain or variable region thereof.

In one embodiment, the nucleic acid molecule encodes an antibody light chain or variable region thereof.

By "nucleic acid molecule" we include DNA (e.g. genomic DNA or complementary DNA) and mRNA molecules, which may be single- or double-stranded. By "isolated" we mean that the nucleic acid molecule is not located or otherwise provided within a cell.

In one embodiment, the nucleic acid molecule is a cDNA molecule.

It will be appreciated by persons skilled in the art that the nucleic acid molecule may be codon-optimised for expression of the antibody polypeptide in a particular host cell, e.g. for expression in human cells (for example, see Angov, 2011, the disclosures of which are incorporated herein by reference).

Also included within the scope of the invention are the following:

(a) a fifth aspect of the invention provides a vector (such as an expression vector) comprising a nucleic acid molecule according to the fourth aspect of the invention;

(b) a sixth aspect of the invention provides a host cell (such as a mammalian cell, *e.g.* human cell, or Chinese hamster ovary cell, *e.g.* CHOK1SV cells) comprising a nucleic acid molecule according to the fourth aspect of the invention or a vector according to the fifth aspect of the invention; and

(c) a seventh aspect of the invention provides a method of making a polypeptide according to the first, second or third aspect of the invention comprising culturing a population of host cells according to the sixth aspect of the invention under conditions in which said polypeptide is expressed, and isolating the polypeptide therefrom.

Methods of production

In an eighth aspect, the present invention provides compositions comprising molecules of the invention, such as the antibodies, bispecific polypeptides, polypeptides, polynucleotides, vectors and cells described herein. For example, the invention provides a composition comprising one or more molecules of the invention, such as one or more antibodies and/or bispecific polypeptides of the invention, and at least one pharmaceutically acceptable carrier.

It will be appreciated by persons skilled in the art that additional compounds may also be included in the pharmaceutical compositions, including, chelating agents such as EDTA, citrate, EGTA or glutathione.

The pharmaceutical compositions may be prepared in a manner known in the art that is sufficiently storage stable and suitable for administration to humans and animals. For example, the pharmaceutical compositions may be lyophilised, *e.g.* through freeze drying, spray drying, spray cooling, or through use of particle formation from supercritical particle formation.

By "pharmaceutically acceptable" we mean a non-toxic material that does not decrease the effectiveness of the CD40 and CEA-binding activity of the bispecific polypeptide of the invention. Such pharmaceutically acceptable buffers, carriers or excipients are well-known in the art (see Remington's Pharmaceutical Sciences, 18th edition, A.R Gennaro, Ed., Mack Publishing Company (1990) and handbook of Pharmaceutical Excipients, 3rd edition, A. Kibbe, Ed ., Pharmaceutical Press (2000), the disclosures of which are incorporated herein by reference).

The term "buffer" is intended to mean an aqueous solution containing an acid-base mixture with the purpose of stabilising pH. Examples of buffers are Trizma, Bicine, Tricine, MOPS, MOPSO, MOBS, Tris, Hepes, HEPBS, MES, phosphate, carbonate, acetate, citrate, glycolate, lactate, borate, ACES, ADA, tartrate, AMP, AMPD, AMPSO, BES, CABS, cacodylate, CHES, DIPSO, EPPS, ethanolamine, glycine, HEPPSO, imidazole, imidazolelactic acid, PIPES, SSC, SSPE, POPSO, TAPS, TABS, TAPSO and TES.

The term "diluent" is intended to mean an aqueous or non-aqueous solution with the purpose of diluting the polypeptide in the pharmaceutical preparation. The diluent may be one or more of saline, water, polyethylene glycol, propylene glycol, ethanol or oils (such as safflower oil, corn oil, peanut oil, cottonseed oil or sesame oil).

The term "adjuvant" is intended to mean any compound added to the formulation to increase the biological effect of the polypeptide of the invention. The adjuvant may be one or more of zinc, copper or silver salts with different anions, for example, but not limited to fluoride, chloride, bromide, iodide, thiocyanate, sulfite, hydroxide, phosphate, carbonate, lactate, glycolate, citrate, borate, tartrate, and acetates of different acyl composition. The adjuvant may also be cationic polymers such as cationic cellulose ethers, cationic cellulose esters, deacetylated hyaluronic acid, chitosan, cationic dendrimers, cationic synthetic polymers such as poly(vinyl imidazole), and cationic polypeptides such as polyhistidine, polylysine, polyarginine, and peptides containing these amino acids.

The excipient may be one or more of carbohydrates, polymers, lipids and minerals. Examples of carbohydrates include lactose, glucose, sucrose, mannitol, and cyclodextrines, which are added to the composition, *e.g.* for facilitating lyophilisation. Examples of polymers are starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polysulphonate, polyethyleneglycol/polyethylene oxide, polyethyleneoxide/polypropylene oxide copolymers, polyvinylalcohol/polyvinylacetate of different degree of hydrolysis, and polyvinylpyrrolidone, all of different molecular weight, which are added to the composition, *e.g.*, for viscosity control, for achieving bioadhesion, or for protecting the lipid from chemical and proteolytic degradation. Examples of lipids are fatty acids, phospholipids, mono-, di-, and triglycerides, ceramides, sphingolipids and glycolipids, all of different acyl chain length and saturation, egg lecithin, soy lecithin, hydrogenated egg and soy lecithin, which are added to the composition for reasons similar to those for polymers. Examples of minerals are talc, magnesium oxide, zinc oxide and titanium oxide, which are added to the composition to obtain benefits such as reduction of liquid accumulation or advantageous pigment properties.

The polypeptides of the invention may be formulated into any type of pharmaceutical composition known in the art to be suitable for the delivery thereof.

In one embodiment, the pharmaceutical compositions of the invention may be in the form of a liposome, in which the polypeptide is combined, in addition to other pharmaceutically acceptable carriers, with amphipathic agents such as lipids, which exist in aggregated forms as micelles, insoluble monolayers and liquid crystals. Suitable lipids for liposomal formulation include, without limitation, monoglycerides, diglycerides, sulfatides, lysolecithin, phospholipids, saponin, bile acids, and the like. Suitable lipids also include the lipids above modified by poly(ethylene glycol) in the polar headgroup for prolonging bloodstream circulation time. Preparation of such liposomal formulations can be found in for example US 4,235,871, the disclosures of which are incorporated herein by reference.

The pharmaceutical compositions of the invention may also be in the form of biodegradable microspheres. Aliphatic polyesters, such as poly(lactic acid) (PLA), poly(glycolic acid) (PGA), copolymers of PLA and PGA (PLGA) or poly(caprolactone) (PCL), and polyanhydrides have been widely used as biodegradable polymers in the production of microspheres. Preparations of such microspheres can be found in

US 5,851,451 and in EP 0 213 303, the disclosures of which are incorporated herein by reference.

In a further embodiment, the pharmaceutical compositions of the invention are provided in the form of polymer gels, where polymers such as starch, cellulose ethers, cellulose carboxymethylcellulose, hydroxypropylmethyl cellulose, hydroxyethyl cellulose, ethylhydroxyethyl cellulose, alginates, carageenans, hyaluronic acid and derivatives thereof, polyacrylic acid, polyvinyl imidazole, polysulphonate, polyethyleneglycol/ polyethylene oxide, polyethyleneoxide/polypropylene oxide copolymers, polyvinylalcohol/ polyvinylacetate of different degree of hydrolysis, and polyvinylpyrrolidone are used for thickening of the solution containing the agent. The polymers may also comprise gelatin or collagen.

Alternatively, the polypeptide may simply be dissolved in saline, water, polyethylene glycol, propylene glycol, ethanol or oils (such as safflower oil, corn oil, peanut oil, cottonseed oil or sesame oil), tragacanth gum, and/or various buffers.

It will be appreciated that the pharmaceutical compositions of the invention may include ions and a defined pH for potentiation of action of the active polypeptide. Additionally, the compositions may be subjected to conventional pharmaceutical operations such as sterilisation and/or may contain conventional adjuvants such as preservatives, stabilisers, wetting agents, emulsifiers, buffers, fillers, *etc*.

The pharmaceutical compositions according to the invention may be administered via any suitable route known to those skilled in the art. Thus, possible routes of administration include parenteral (intravenous, subcutaneous, and intramuscular), topical, ocular, nasal, pulmonar, buccal, oral, parenteral, vaginal and rectal. Also administration from implants is possible.

In one preferred embodiment, the pharmaceutical compositions are administered parenterally, for example, intravenously, intracerebroventricularly, intraarticularly, intra-arterially, intraperitoneally, intrathecally, intraventricularly, intrasternally, intracranially, intramuscularly or subcutaneously, or they may be administered by infusion techniques. They are conveniently used in the form of a sterile aqueous solution which may contain other substances, for example, enough salts or glucose to make the solution isotonic with blood. The aqueous solutions should be suitably buffered (preferably to a pH of from 3 to 9), if necessary. The preparation of suitable

parenteral formulations under sterile conditions is readily accomplished by standard pharmaceutical techniques well known to those skilled in the art.

Formulations suitable for parenteral administration include aqueous and non-aqueous sterile injection solutions which may contain anti-oxidants, buffers, bacteriostats and solutes which render the formulation isotonic with the blood of the intended recipient; and aqueous and non-aqueous sterile suspensions which may include suspending agents and thickening agents. The formulations may be presented in unit-dose or multi-dose containers, for example sealed ampoules and vials, and may be stored in a freeze-dried (lyophilised) condition requiring only the addition of the sterile liquid carrier, for example water for injections, immediately prior to use. Extemporaneous injection solutions and suspensions may be prepared from sterile powders, granules and tablets of the kind previously described.

Thus, the pharmaceutical compositions of the invention are particularly suitable for parenteral, *e.g.* intravenous, administration.

Alternatively, the pharmaceutical compositions may be administered intranasally or by inhalation (for example, in the form of an aerosol spray presentation from a pressurised container, pump, spray or nebuliser with the use of a suitable propellant, such as dichlorodifluoromethane, trichlorofluoro-methane, dichlorotetrafluoro-ethane, a hydrofluoroalkane such as 1,1,1,2-tetrafluoroethane (HFA 134A3 or 1,1,1,2,3,3,3-heptafluoropropane (HFA 227EA3), carbon dioxide or other suitable gas). In the case of a pressurised aerosol, the dosage unit may be determined by providing a valve to deliver a metered amount. The pressurised container, pump, spray or nebuliser may contain a solution or suspension of the active polypeptide, *e.g.* using a mixture of ethanol and the propellant as the solvent, which may additionally contain a lubricant, e.g. sorbitan trioleate. Capsules and cartridges (made, for example, from gelatin) for use in an inhaler or insufflator may be formulated to contain a powder mix of a compound of the invention and a suitable powder base such as lactose or starch.

The pharmaceutical compositions will be administered to a patient in a pharmaceutically effective dose. A 'therapeutically effective amount', or 'effective amount', or 'therapeutically effective', as used herein, refers to that amount which provides a therapeutic effect for a given condition and administration regimen. This is a predetermined quantity of active material calculated to produce a desired therapeutic effect in association with the required additive and diluent, *i.e.* a carrier or administration vehicle. Further, it is intended to mean an amount sufficient to reduce

and most preferably prevent, a clinically significant deficit in the activity, function and response of the host. Alternatively, a therapeutically effective amount is sufficient to cause an improvement in a clinically significant condition in a host. As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent. In the methods and use for manufacture of compositions of the invention, a therapeutically effective amount of the active component is provided. A therapeutically effective amount can be determined by the ordinary skilled medical or veterinary worker based on patient characteristics, such as age, weight, sex, condition, complications, other diseases, *etc.*, as is well known in the art. The administration of the pharmaceutically effective dose can be carried out both by single administration in the form of an individual dose unit or else several smaller dose units and also by multiple administrations of subdivided doses at specific intervals. Alternatively, the dose may be provided as a continuous infusion over a prolonged period.

Particularly preferred compositions are formulated for systemic administration.

The composition may preferably be formulated for sustained release over a period of time. Thus the composition may be provided in or as part of a matrix facilitating sustained release. Preferred sustained release matrices may comprise a montanide or γ-polyglutamic acid (PGA) nanoparticles.

The bispecific polypeptides can be formulated at various concentrations, depending on the efficacy/toxicity of the polypeptide being used. For example, the formulation may comprise the active polypeptide at a concentration of between 0.1 μM and 1 mM, more preferably between 1 μM and 500 μM, between 500 μM and 1 mM, between 300 μM and 700 μM, between 1 μM and 100 μM, between 100 μM and 200 μM, between 200 μM and 300 μM, between 300 μM and 400 μM, between 400 μM and 500 μM, between 500 μM and 600 μM, between 600 μM and 700 μM, between 800 μM and 900 μM or between 900 μM and 1 mM. Typically, the formulation comprises the active polypeptide at a concentration of between 300 μM and 700 μM.

Typically, the therapeutic dose of the bispecific polypeptide (with or without a therapeutic moiety) in a human patient will be in the range of 100 μg to 700 mg per administration (based on a body weight of 70 kg). For example, the maximum therapeutic dose may be in the range of 0.1 to 20 mg/kg per administration, *e.g.*

between 0.1 and 5 mg/kg or between 1 and 5 mg/kg or between 0.1 and 2 mg/kg. It will be appreciated that such a dose may be administered at different intervals, as determined by the oncologist/physician; for example, a dose may be administered daily, twice-weekly, weekly, bi-weekly or monthly.

It will be appreciated by persons skilled in the art that the pharmaceutical compositions of the invention may be administered alone or in combination with other therapeutic agents used in the treatment of cancers and/or tumours, such as antimetabolites, alkylating agents, anthracyclines and other cytotoxic antibiotics, vinca alkyloids, etoposide, platinum compounds, taxanes, topoisomerase I inhibitors, other cytostatic drugs, antiproliferative immunosuppressants, antiangiogenic drugs, cancer vaccines, adoptive cell transfer (T cells/DC, NK cells), corticosteroids, sex hormones and hormone antagonists, and other therapeutic antibodies (such as antibodies against a CEA or an immune checkpoint modulator).

For example, the pharmaceutical compositions of the invention may be administered in combination with an immunotherapeutic agent that binds a target selected from the group consisting of PD-1/PD-L1, VGFR, EGFR, HER2, CTLA-4, CD137, OX40, GITR, LAG3, TIM3, CD27, VISTA and KIR.

Thus, the invention encompasses combination therapies comprising a bispecific polypeptide of the invention together with a further immunotherapeutic agent, effective in the treatment of cancer and/or a tumour, which specifically binds to an immune checkpoint molecule. It will be appreciated that the therapeutic benefit of the further immunotherapeutic agent may be mediated by attenuating the function of an inhibitory immune checkpoint molecule and/or by activating the function of a stimulatory immune checkpoint or co-stimulatory molecule.

In one embodiment, the further immunotherapeutic agent is selected from the group consisting of:

(a) an immunotherapeutic agent that inhibits the function of PD-1 and/or PD-L1;
(b) an immunotherapeutic agent that inhibits the function of CTLA-4;
(c) an immunotherapeutic agent that activates the function of CD137;
(d) an immunotherapeutic agent that binds activates the function of OX40;
(e) an immunotherapeutic agent that inhibits the function of LAG3;
(f) an immunotherapeutic agent that inhibits the function of TIM3;
(g) an immunotherapeutic agent that inhibits the function of VISTA;

(h) an immunotherapeutic agent that inhibits the function of VGFR;

(i) an immunotherapeutic agent that inhibits the function of EGFR; and

(j) an immunotherapeutic agent that inhibits the function of HER2.

Thus, the further immunotherapeutic agent may be a PD-1 inhibitor, such as an anti-PD-1 antibody, or antigen-binding fragment thereof capable of inhibiting PD-1 function (for example, Nivolumab, Pembrolizumab, Lambrolizumab, PDR-001, MEDI-0680 and AMP-224). Alternatively, the PD-1 inhibitor may comprise or consist of an anti-PD-L1 antibody, or antigen-binding fragment thereof capable of inhibiting PD-1 function (for example, Durvalumab, Atezolizumab, Avelumab and MDX-1105).

In another embodiment, the further immunotherapeutic agent is a CTLA-4 inhibitor, such as an anti-CTLA-4 antibody or antigen-binding portion thereof.

In a further embodiment, the further immunotherapeutic agent activates CD137, such as an agonistic anti-CD137 antibody or antigen-binding portion thereof.

In a further embodiment, the further immunotherapeutic agent activates OX40, such as an agonistic anti-OX40 antibody or antigen-binding portion thereof.

In a further embodiment, the further immunotherapeutic agent inhibits the function of LAG3, TIM3 or VISTA (Lines *et al.* 2014).

In another embodiment, the further immunotherapeutic agent is a VGFR inhibitor, such as an anti-VGFR antibody or antigen-binding portion thereof.

In a further embodiment, the further immunotherapeutic agent activates EGFR, such as an agonistic anti-EGFR antibody or antigen-binding portion thereof.

In a further embodiment, the further immunotherapeutic agent activates HER2, such as an agonistic anti-HER2 antibody or antigen-binding portion thereof.

It will be appreciated by persons skilled in the art that the presence of the two active agents (as detailed above) may provide a synergistic benefit in the treatment of a tumour in a subject. By "synergistic" we include that the therapeutic effect of the two agents in combination (*e.g.* as determined by reference to the rate of growth or the size of the tumour) is greater than the additive therapeutic effect of the two agents

administered on their own. Such synergism can be identified by testing the active agents, alone and in combination, in a relevant cell line model of the solid tumour.

Also within the scope of the present invention are kits comprising polypeptides or other compositions of the invention and instructions for use. The kit may further contain one or more additional reagents, such as an additional therapeutic or prophylactic agent as discussed above.

Medical uses and methods

The polypeptides in accordance with the present invention may be used in therapy or prophylaxis. In therapeutic applications, polypeptides or compositions are administered to a subject already suffering from a disorder or condition, in an amount sufficient to cure, alleviate or partially arrest the condition or one or more of its symptoms. Such therapeutic treatment may result in a decrease in severity of disease symptoms, or an increase in frequency or duration of symptom-free periods. An amount adequate to accomplish this is defined as "therapeutically effective amount". In prophylactic applications, polypeptides or compositions are administered to a subject not yet exhibiting symptoms of a disorder or condition, in an amount sufficient to prevent or delay the development of symptoms. Such an amount is defined as a "prophylactically effective amount". The subject may have been identified as being at risk of developing the disease or condition by any suitable means.

Thus, a ninth aspect of the invention provides a bispecific polypeptide according to the first or third aspect of the invention or polypeptide according to the second aspect of the invention for use in medicine.

A tenth aspect of the invention provides a bispecific polypeptide according to the first or third aspect of the invention or polypeptide according to the second aspect of the invention for use in treating cancer and/or a tumour and/or a non-cancer condition in a subject. A cancer and/or a tumour may be referred to as a neoplastic disorder.

An eleventh aspect of the invention provides a use of a bispecific polypeptide according to the first or third aspect of the invention or polypeptide according to the second aspect of the invention in the preparation of a medicament for treating cancer and/or a tumour and/or a non-cancer condition in a subject.

In one embodiment, the bispecific polypeptide or polypeptide for the use of the tenth aspect of the invention, or the use of the eleventh aspect of the invention, is for use in combination with one or more additional therapeutic agents.

In one embodiment, the one or more additional therapeutic agents is/are an immunotherapeutic agent that binds a target selected from the group consisting of PD-1/PD-L1, VGFR, EGFR, HER2, CTLA-4, CD137, OX40, GITR, LAG3, TIM3, CD27, VISTA and KIR.

In one embodiment, the bispecific polypeptide or polypeptide for the use of the tenth aspect of the invention, or the use of the eleventh aspect of the invention, is for administration systemically.

A twelfth aspect of the invention provides a method for the treatment and/or diagnosis of cancer and/or a tumour and/or a non-cancer condition in a subject, comprising the step of administering to the subject an effective amount of a bispecific polypeptide according to the first or third aspect of the invention or polypeptide according to the second aspect of the invention.

An alternative twelfth aspect of the invention, invention provides an *in vitro* method for the diagnosis of cancer and/or a tumour, comprising using a according to the first or third aspect of the invention or polypeptide according to the second aspect of the invention.

In one embodiment, the method comprises administering the bispecific polypeptide systemically.

In one embodiment, the methods further comprise administering to the subject one or more additional therapeutic agents.

By 'treatment' we include both therapeutic and prophylactic treatment of the patient. The term 'prophylactic' is used to encompass the use of an agent, or formulation thereof, as described herein which either prevents or reduces the likelihood of a neoplastic disorder, or the spread, dissemination, or metastasis of cancer cells in a patient or subject. The term 'prophylactic' also encompasses the use of an agent, or formulation thereof, as described herein to prevent recurrence of a neoplastic disorder in a patient who has previously been treated for the cancer and/or tumour.

Preferably, the cancer and/or the tumour is a cancer and/or the tumour associated with CEA; for example, CEA expression. By "associated with CEA", we include that the CEA is cancer and/or the tumour is caused by CEA and/or CEA is a marker for the cancer and/or the tumour.

In one embodiment, the cancer and/or the tumour comprises target cells comprising expression of CEA.

Preferably, the expression of CEA is an intermediate level of CEA expression or a high level of CEA expression.

In one embodiment, the intermediate level of CEA expression is characterised by the target cell expressing about 10,000 or more CEA receptors per target cell; for example, about 11,000 or more; about 12,000 or more; about 13,000 or more; about 14,000 or more; about 15,000 or more; about 16,000 or more; about 17,000 or more; about 18,000 or more; about 19,000 or more; about 20,000 or more; about 25,000 or more; about 30,000 or more; about 35,000 or more; about 40,000 or more; about 50,000 or more; about 60,000 or more; about 70,000 or more; about 80,000 or more; about 90,000 or more; about 100,000 or more; about 125,000 or more; about 150,000 or more; or about 175,000 or more CEA receptors per target cell. In another embodiment, the intermediate level of CEA expression is characterised by the target cell expressing about 10,000 to about 200,000 CEA receptors per target cell; for example, about 20,000 to about 175,000 CEA receptors per target cell or 20,000 to about 200,000 CEA receptors per target cell or about 50,000 to about 175,000 CEA receptors per target cell or about 50,000 to about 200,000 CEA receptors per target cell. Preferably, the CEA receptors are CEACAM5 receptors.

In one embodiment, the high level of CEA expression is characterised by the target cell expressing about 200,000 or more CEA receptors per target cell; for example, about 225,000 or more; about 250,000 or more; about 275,000 or more; about 300,000 or more; about 325,000 or more; about 350,000 or more; about 375,000 or more; about 400,000 or more; about 425,000 or more; about 450,000 or more; about 475,000 or more; about 500,000 or more; about 600,000 or more; about 700,000 or more; about 800,000 or more; about 900,000 or more; or about 1,000,000 CEA receptors per target cell, preferably about 300,000 of more CEA receptors per target cell. In another embodiment, the high level of CEA expression is characterised by the target cell expressing about 200,000 to about 1,000,000 CEA receptors per target cell; for example, about 200,000 to about 500,000 CEA receptors per target cell or about

300,000 to about 500,000 CEA receptors per target cell. Preferably, the CEA receptors are CEACAM5 receptors.

In one embodiment, the cancer and/or the tumour does not comprise a cell with no or a low level of CEA expression. In one embodiment, the low level of CEA expression is characterised by the cell expressing about 10,000 or fewer CEA receptors per target cell; for example, about 9,000 or fewer; about 8,000 or fewer; about 7,000 or fewer; about 6,000 or fewer; about 5,000 or fewer; about 4,000 or fewer; about 3,000 or fewer; about 2,000 or fewer; or about 1,000 or fewer CEA receptors per cell.

In one embodiment, the CEA is a tumor-associated CEA.

Preferably, the CEA is a CEACAM.

In one embodiment, the CEACAM is one or more selected from the listing consisting of: CEACAM1; CEACAM3; CEACAM4; CEACAM5; CEACAM6; CEACAM7; CEACAM8; CEACAM16; CEACAM18; CEACAM19; CEACAM20; and CEACAM21. It will be appreciated that the reference to the aforementioned CEACAM molecules includes splice variants.

Preferably, the CEACAM is one or more selected from the listing consisting of: CEACAM1; CEACAM5; and CEACAM6. Preferably, the CEACAM is CEACAM1. Most preferably, the CEACAM is CEACAM5.

In a preferred embodiment, B2 is capable of specifically binding to CEACAM5 but not other CEACAMs, particularly not CEACAM1.

In one embodiment, the cancer and/or the tumour is one or more cancer and/or tumour selected from the list consisting of: prostate cancer and/or a prostate tumour; breast cancer and/or a breast tumour; lung cancer and/or a lung tumour; colorectal cancer and/or a colorectal tumour; melanomas; bladder cancer and/or a bladder tumour; brain/CNS cancer and/or a brain/CNS tumour; cervical cancer and/or a cervical tumour; oesophageal cancer and/or a oesophageal tumour; gastric cancer and/or a gastric tumour; head/neck cancer and/or a head/neck tumour; kidney cancer and/or a kidney tumour; liver cancer and/or a liver tumour; a carcinoma; leukaemia; lymphomas; ovarian cancer and/or an ovarian tumour; pancreatic cancer and/or a pancreatic tumour; tonsil cancer and/or a tonsil tumour; and sarcomas. Preferably, a carcinoma.

Preferably, the one or more cancer and/or tumour selected from the list consisting of: breast cancer and/or a breast tumour; lung cancer and/or a lung tumour; colorectal cancer and/or a colorectal tumour; gastric cancer and/or a gastric tumour; and/or pancreatic cancer and/or a pancreatic tumour.

In a preferred embodiment, the cancer and/or tumour is a colorectal cancer and/or a colorectal tumour.

In a preferred embodiment, the cancer and/or tumour is a tonsil cancer and/or a tonsil tumour.

Preferably, the carcinoma is one or more carcinoma selected from the listing consisting of: gastric carcinoma; oesophageal carcinoma; colorectal carcinoma; pancreatic carcinoma; lung carcinoma; breast carcinoma; cervical carcinoma; cholangiocarcinoma; and medullary thyroid carcinoma.

In a preferred embodiment, the carcinoma is a colorectal carcinoma.

Preferably, the tumour is a solid tumour.

In one embodiment, the non-cancer condition is a non-cancer condition is one associated with CEA; for example, CEA expression. By "associated with CEA", we include that the CEA is non-cancer condition is caused by CEA and/or CEA is a marker for the non-cancer condition.

In one embodiment, the non-cancer condition comprises target cells comprising expression of CEA.

Preferably, the expression of CEA is an intermediate level of CEA expression or a high level of CEA expression, as discussed herein.

Preferably, the one or more non-cancer condition is selected from the list consisting of: ulcerative colitis, pancreatitis; cirrhosis; COPD; Crohn's disease; and/or hypothyroidism.

In one embodiment, the subject is human.

Optimised RUBY™ format

A thirteenth aspect of the invention provides a bispecific polypeptide comprising the optimised RUBY™ format, preferably wherein the bispecific polypeptide has specificity for a first antigen and a second antigen.

In a particular embodiment, the bispecific polypeptide comprises:

(a) an immunoglobulin molecule having specificity for a first antigen, the immunoglobulin molecule comprising a first heavy chain polypeptide and a first light chain polypeptide; and

(b) at least one Fab fragment having specificity for a second antigen, the Fab fragment comprising a second heavy chain polypeptide and a second light chain polypeptide

wherein the second light chain polypeptide is fused to the C-terminus of the first heavy chain polypeptide

and wherein the bispecific antibody comprises one or more mutations discussed in relation to the optimised RUBY™ format to promote association of the polypeptide; in particular, of the first heavy chain polypeptide with the first light chain polypeptide and/or to promote association of the second heavy chain polypeptide with the second light chain polypeptide.

All of the features of the optimised RUBY™ format, and the necessary associated embodiments of the RUBY™ format, as discussed for the first aspect of the invention are relevant to, and included in, the thirteenth aspect of the invention. In particular, the features discussed below.

The optimised RUBY™ format has the structure shown in Figure 23 with further optimised mutations, when compared to the RUBY™ format. As will be appreciated by the skilled person, technology relating to antibody format has wide applicability to a wide range of different target antigens.

Although bispecific polypeptides in the "RUBY™ format" can be reproducibly produced with an excellent level of purity, bispecific polypeptides in the "optimised RUBY™ format" can be reproducibly produced at an even higher level of purity. Further,

bispecific polypeptides in the "optimised RUBY™ format" have been engineered to carry a reduced risk of provoking immunogenic responses directed against the bispecific polypeptide itself.

In one embodiment of the thirteenth aspect of the invention, the bispecific polypeptide is a bispecific antibody.

In one embodiment, the bispecific polypeptide comprises an immunoglobulin arranged as an antibody with two arms and therefore two binding sites for the first antigen, and two of the Fab fragments, each providing a binding site for the second antigen. Thus, there are two binding sites for the first antigen and two binding sites for the second antigen. In a preferred embodiment of the thirteenth aspect of the invention, the first antigen and/or second antigen are not CD40 and/or CEA. In a further preferred embodiment of the thirteenth aspect of the invention the first antigen and/or second antigen are a protein and/or peptide that is not CD40 and/or CEA.

In one embodiment, the one or more Fab fragment(s) is linked to the C-terminal end of the immunoglobulin via a linker.

In one embodiment of the first aspect of the invention, the bispecific polypeptide is tetravalent, capable of binding bivalently to each of the two antigens.

The optimised mutations are described below as "optimised mutation set 1" and "optimised mutation set 2" – including "set 2a" and/or "set 2b". It will be appreciated by the skilled person various combinations of these optimised mutations could be used in a bispecific polypeptide of the invention, as well as in combination with any of the "RUBY™ format" mutations described above. It will also be appreciated that the variations of those mutations as described herein would also work as part of the invention. All mutations in variable domains (VH or VL) are numbered according to the IMGT numbering system, and all mutations in the constant domains are numbered according to the EU numbering system.

Mutation set 1 - Mutations in the variable domain heavy (VH):
T65E, T65A, T65I.

Mutation set 2 - any individual and/or any combination of the mutations listed in set 2a and set 2b. Set 2a - mutations in the CH1: Y180A, Y180G, Y180I, Y180N, Y180S, Y180T, Y180V, or Y180W, and/or S183N or S183T, and/or V188G; preferably, Y180T.

Set 2b - mutations in the CKappa domain: A111R, A111T, A111W or A111V, and/or T109P; preferably: T109P and/or A111V; and/or mutations in the variable domain light (VL): I126A, I126G, I126H, I126N, I126P, I126Q, I126S, or I126T.

In one embodiment of the invention the mutations are at positions selected from the group consisting of:

(a) the T65 position in the VH (according to the IMGT numbering system); and/or
(b) one or more of the following positions in the CH1: Y180; S183; and V188, preferably Y180 (according to the EU numbering system); and/or
(c) one or more of the following positions in the CKappa domain: A111 and T109 (according to the EU or Kabat numbering systems); and/or
(d) the I126 position in the VL (according to the IMGT numbering system).

In a particular embodiment, the mutation is at the T65 position in the variable domain heavy (VH) (according to the IMGT numbering system).

In a particular embodiment, the mutations are one or more of the following positions in the CH1: Y180; S183; and V188, preferably Y180 (according to the EU numbering system).

In a particular embodiment, the mutations are one or more of the following positions in the CKappa domain: A111 and T109 (according to the EU or Kabat numbering systems); and/or the I126 position in the VL (according to the IMGT numbering system).

In one embodiment of the invention the mutations are selected from the group consisting of:

(a) X65E/A/I in the VH (according to the IMGT numbering system); and/or
(b) one or more of the following mutations in the CH1: X180A/G/I/N/S/T/V/W; X183N/T; and X188G; preferably, X180T (according to the EU numbering system); and/or
(c) one or more of the following mutations in the C-Kappa domain: X111R/T/W/V; and X109P, preferably X111V and X109P (according to the EU or Kabat numbering systems); and/or
(d) X126A/G/H/N/P/Q/S/T in the VL (according to the IMGT numbering system).
*X refers to any amino acid

In a particular embodiment, the mutation is X65E/A/I in the VH chain (according to the IMGT numbering system).
*X refers to any amino acid

In a particular embodiment, the mutation is one or more of the following mutations in the CH1: X180A/G/I/N/S/T/V/W; X183N/T; and X188G; preferably, X180T (according to the EU numbering system).
*X refers to any amino acid

In a particular embodiment, the mutation is one or more of the following mutations in the CKappa domain: X111R/T/W/V; and X109P, preferably X111V and X109P (according to the IMGT numbering system); and/or the mutation is X126A/G/H/N/P/Q/S/T in the VL (according to the IMGT numbering system).
*X refers to any amino acid

For example, the mutations may be selected from the group consisting of:

(a) one or more of the following mutations in the VH: T65E; T65A; and T65I (according to the IMGT numbering system); and/or
(b) one or more of the following mutations in the CH1: Y180A; Y180G; Y180I; Y180N; Y180S; Y180T; Y180V; Y180W; S183N; S183T; V188G, preferably Y180T (according to the EU numbering system); and/or
(c) one or more of the following mutations in the CKappa domain: A111R; A111T; A111W; A111V; and T109P, preferably T109P and A111V (according to the EU numbering system); and/or
(d) one or more of the following mutations in the VL: I126A; I126G; I126H; I126N; I126P; I126Q; I126S; and I126T (according to the IMGT numbering system).

In a particular example, the mutations are one or more of the following mutations in the VH: T65E; T65A; and T65I (according to the IMGT numbering system).

In a particular example, the mutations are one or more of the following mutations in the CH1: Y180A; Y180G; Y180I; Y180N; Y180S; Y180T; Y180V; Y180W; S183N; S183T; V188G, preferably Y180T (according to the EU numbering system).

In a particular example, the mutations are one or more of the following mutations in the C-kappa domain: A111R; A111T; A111W; A111V; and T109P, preferably T109P

and A111V (according to the EU or Kabat numbering systems); and/or one or more of the following mutations in the VL: I126A; I126G; I126H; I126N; I126P; I126Q; I126S; and I126T (according to the IMGT numbering system).

As discussed above, any combination of the "RUBY™ format" mutations and "optimised RUBY™ format" mutations can be used in the same bispecific antibody, such as any one or more of the following "RUBY™ format" mutations in (a) to (d), or variations described herein, being combined with any one or more of the following "optimised RUBY™ format" mutations in (e) to (g), or variations described herein:

(a) one or more of the following mutations in the CH1 domain: H168A, F170G and/or T187E (according to EU numbering system);
(b) one or more of the following mutations in the CKappa domain: L135Y, S176W, S114A and/or N137K (according to EU or Kabat numbering systems) and/or one or more of the following mutations in the CLambda domain: L135Y, S176W, T114A and/or S137K (according to Kabat numbering system);
(c) mutations in the VL: Q44R or Q44E (according to IMGT numbering system); ad
(d) mutations in the VH: Q44E or Q44R (according to IMGT numbering system);
(e) mutations in the VH: T65E, T65A or T65I (according to IMGT numbering system);
(f) mutation in the CH1: Y180T (according to EU numbering system); and/or
(g) mutations in the CKappa: T109P and/or A111V (according to EU numbering system).

Accordingly, in a particular embodiment, a bispecific antibody with combined "RUBY™ format" mutations and "optimised RUBY™ format" mutations could include the following mutations:

- one or more of the following mutations in the CH1 domain: H168A, F170G, Y180T and/or T187E (according to EU numbering system);
- one or more of the following mutations in the CKappa domain: T109P, A111V, L135Y, S176W, S114A and/or N137K (according to EU or Kabat numbering systems) and/or one or more of the following mutations in the CLambda domain: L135Y, S176W, T114A and/or S137K (according to Kabat numbering system);
- mutations in the VL: Q44R or Q44E (according to IMGT numbering system); and/or
- one or more of the following mutations in the VH: Q44E or Q44R, and/or T65E, T65A or T65I (according to IMGT numbering system).

The listing or discussion of an apparently prior-published document in this specification should not necessarily be taken as an acknowledgement that the document is part of the state of the art or is common general knowledge.

The use of the word "a" or "an" when used in conjunction with the term "comprising" in the claims and/or the specification may mean "one," but it is also consistent with the meaning of "one or more," "at least one," and "one or more than one."

These, and other, embodiments of the invention will be better appreciated and understood when considered in conjunction with the above description and the accompanying drawings. It should be understood, however, that the above description, while indicating various embodiments of the invention and numerous specific details thereof, is given by way of illustration and not of limitation. Many substitutions, modifications, additions and/or rearrangements may be made within the scope of the invention without departing from the spirit thereof, and the invention includes all such substitutions, modifications, additions and/or rearrangements.

The following drawings form part of the present specification and are included to further demonstrate certain aspects of the present invention. The invention may be better understood by reference to one or more of these drawings in combination with the detailed description of specific embodiments presented herein.

Brief description of figures

Preferred, non-limiting examples which embody certain aspects of the invention will now be described, with reference to the following figures:

Figure 1. ELISA analysis of 22 CEA antibodies isolated using phage displayed combined with next generation sequencing. Binders in IgG1 format were analysed for binding to human CEACAM5 (abbreviated CAM5) (diagonal bars) or human CEACAM1 (abbreviated CAM1) (back bars).

Figure 2. Ability of CD40 and CEACAM5 targeting RUBY™ bsAbs to bind both antigens simultaneously, as measured by dual target ELISA. Panel A shows binding curves for Multi34, Multi35, Multi37 and Multi38. Panel B shows binding curves for Multi39, Multi40, Multi41 and Multi42, Panel C shows binding curves for Multi44, Multi45, Multi46 and Multi47. Lastly, Panel D shows binding curves for Multi48, Multi4 and AC_05339.

Figure 3. Cross-reactivity of RUBY™ bsAbs (Mult34, Multi35, Multi37-Multi42, Multi44-Multi49) with CEA protein family members CEACAM1, 5, 6 and 8, evaluated in ELISA.

Figure 4. Kinetic measurement in Octet of interaction between captured CD40 CEACAM5 targeting bispecific antibodies (Multi 34, Multi42, Multi46 and AC_5339) against soluble monomeric human CEACAM5 (at varying concentrations ranging between 100-1.6nM). Association was measured for 100 sec followed by dissociation for 100 sec into 1x kinetic buffer.

Figure 5. Kinetic measurement of bispecific antibodies (at varying concentrations ranging between 50-0.8nM) in solution interacting with captured human CEACAM5-biotin in Octet. Association was measured for 100 sec followed by dissociation for 100 sec into kinetic buffer.

Figure 6. Binding of CD40-CEACAM5 bispecific antibodies to CEACAM5-transfected CHO cells. Binding of CD40-CEA bispecific antibodies was detected by flow cytometry using fluorochrome-conjugated anti-human IgG. Panel A shows binding curves for Multi34 and Multi35. Panel B shows binding curves for Multi41, Multi42, Multi44 and ffAC_5339. Panel C shows binding curves for Multi46, Multi47, Multi48 and Multi49. Panel D shows binding curves for ffAC_5337 and AC_5339. Panel E shows binding curves for AC_05355 and AC_05339.

Figure 7. Binding of CD40-CEACAM5 bispecific antibodies to CEACAM1-transfected CHO cells (A-D) and to CHO wt cells (F). Binding of CD40-CEACAM5 bispecific antibodies was detected by flow cytometry using fluorochrome-conjugated anti-human IgG.

Figure 8. Binding of CD40-CEACAM5 bispecific antibodies to CEACAM5 expressing tumor cells. The tumor cell line MKN45, expressing high levels of *CEACAM5*A-C), LS174T expressing intermediate levels of CEACAM5 (D-E), and Lovo expressing low levels CEACAM5 (G-J). Binding of CD40-CEACAM5 bispecific antibodies was detected by flow cytometry using fluorochrome-conjugated anti-human IgG.

Figure 9. Effect of the CD40-CEACAM5 bispecific antibodies on CD40 reporter cells cultured with titrated antibodies in the presence or absence of CEACAM5 expressed on CHO cells. The response was calculated as fold induction to background.

Figure 10. Effect of the CD40-CEACAM5 bispecific antibodies on CD40 reporter cells when co-cultured with tumor cells with different CEACAM5 receptor density. CD40-CEACAM5 containing CD40 clone G12 (A-D), CEACAM5-CD40 containing CD40 clone G12 (E-H) and CD40-CEACAM5 containing CD40 clone 1132 (I-J). The response was calculated as fold induction to background.

Figure 11. Effect of the CD40-CEACAM5 bispecific antibodies on CD40 reporter cells co-cultured with CEACAM5 expressing CHO cells and titrated antibodies in the presence or absence of soluble CEACAM5. The response was calculated as fold induction to background.

Figure 12. Effect of the CD40-CEACAM5 bispecific antibodies on B cell activation. Primary human B cells were cultured with titrated antibodies in the presence or absence of CEACAM5 expressed on CHO cells. After 2 days, expression of CD86 on B cells was analyzed by FACS.

Figure 13. Effect of the CD40-cCEACAM5 bispecific antibody AC_05355 on B cell activation in the presence of human or cynomolgus CEACAM5 transfected cells. Primary human B cells were cultured with titrated antibodies in the presence of human or cynomolgus CEACAM5 expressed on CHO cells. After 2 days, expression of CD86 on B cells was analyzed by FACS. The graph shows pooled results from 6 donors.

Figure 14. BsAb AC_05339 (0.02nM) w/o crosslinking to CHO-CEACAM5 or CHO-wt cells, activating HEK Blue CD40L™ reporter cells in presence of Raji cells after 20h incubation in a Transwell system. Activation monitored as SEAP release into culture medium, measured with QuantiBlue™. Mean +SD of triplicate.

Figure 15. BsAb Multi34 (3-0.004nM, single sample) crosslinked to CHO-CEACAM5 cells activating HEK Blue CD40L™ reporter cells w/o of presence of Raji sink cells in a Transwell system. Activation monitored as release of SEAP into culture media following 20h culture, measured with QuantiBlue™.

Figure 16. BsAb Multi42 (3-0.004nM, single sample) crosslinked to CHO-CEACAM5 cells activating HEK Blue CD40L™ reporter cells w/o of presence of Raji sink cells in a Transwell system. Activation monitored as release of SEAP into culture media following 20h culture, measured with QuantiBlue™.

Figure 17. BsAb Multi46 (100-0.1nM, single sample) crosslinked to CHO-CEACAM5 cells activating HEK Blue CD40L™ reporter cells w/o of presence of Raji sink cells in a Transwell system. Activation monitored as release of SEAP into culture media following 20h culture, measured with QuantiBlue™.

Figure 18. Colocalization of tumor debris and Raji cells. Raji cells were incubated with CEACAM5 expressing MKN45 tumor debris and AC_05339 or the control antibody 1132 with a silenced Fc, more specifically of the IgG1 isotype and carrying the L234A and L235A mutations (the antibody is herein refer to 1132.m2). Images were captured with a Cytation5 live imaging system and the number of tumor debris colocalized with Raji cells after 4 h was analyzed using Gen5 software.

Figure 19. MC38-CEACAM5 tumor growth and MC38-wt rechallenge. hCD40tg mice inoculated with MC38-CEACAM5 tumors were dosed with the indicated treatments on days 7, 10, and 13 post-inoculations. Tumors were frequently measured until the first mouse in any of the treatment groups reached a tumor volume above the ethical limit. Statistical analysis of tumor volumes on day 38 was performed using a Mann-Whitney test (n=10, * $p<0.05$). Naïve control hCD40tg mice or mice cured from MC38-CEACAM5 tumors by treatment with a CD40-CEACAM5 bsAb (complete responders) were inoculated with MC38-wt tumors (rechallenged). Tumors were frequently measured until the first mouse in any of the treatment groups reached a tumor volume above the ethical limit.

Figure 20. Effect of the CD40-CEACAM5 bispecific antibody AC_05355 on B cell activation in the presence of cynomolgus CEACAM5 (cCEACAM5) transfected CHO cells. Primary cynomolgus B cells were cultured with titrated antibodies in the presence cCEACAM5 expressed on CHO cells. After 2 days, expression of CD86 on B cells was analyzed by FACS. The graph shows pooled data from 2 donors.

Figure 21. MC38-CEACAM5 tumor growth. hCD40tg mice inoculated with MC38-CEACAM5 tumors were dosed with the indicated treatments on days 10, 13 and 16 post inoculation. Tumors were frequently measured until the first mouse in any of the treatment groups reached a tumor volume above the ethical limit. Statistical analysis of tumor volumes on day 17 was performed using a Mann-Whitney test (n=10, * $p<0.05$).

Figure 22. This shows a schematic representation of the structure of exemplary formats for a bispecific antibody of the invention. In each format, the constant regions are shown as filled light grey; variable heavy chain regions VH1 are shown as chequered black and white; variable light chain regions VL1 are shown as filled white; variable heavy chain regions VH2 are shown as filled black; and variable light chain regions VL2 are shown as white with diagonal lines. CD40 binding domains (binding domain 1) are typically represented as a pair of a chequered black and white domain with a filled white domain (VH1/VL1); CEA binding domains (binding domain 2) are typically represented as a pair of a filled black domain and a white domain with diagonal lines (VH2/VL2). However, in all of the formats shown, it will be appreciated that binding domains 1 and 2 may be switched. That is, a CD40 binding domain may occur in a position shown in this figure for a CEA-binding domain, and vice versa.

Figure 23. This shows an example composition of a bispecific antibody construct, in the RUBY™ format. The bispecific antibody of Figure 21 is made up of three types of polypeptide chains: (1) IgG heavy chains (white) fused to Fab light chains (chequered) via a polypeptide linker. (2) IgG light chains (bricked) and (3) Fab heavy chains (black). Mutations are introduced in the interface between heavy and light chains.

Figure 24. CD40xTAA bsAbs mediate localization of tumor debris to antigen presenting cells. The number of CEA+ tumor debris clustering with CD40+ cells was quantified after 8 hrs of culture using live cell imaging software. The graphs show the mean (+SD) of duplicate wells in one representative experiment of four (CEA).

Figure 25. Dissociated cells from human colorectal cancer tumors were analyzed for: (left) their CEA-expression (gated on total viable cells), (middle) ability to provide cross-linking to CD40xCEA Neo-X-Prime bsAb in a CD40 reporter assay, and (right) CD83 upregulation following stimulation of the tumor infiltrating immune cells (gated on viable CD45+CD3-CD56- cells) using CD40xCEA Neo-X-Prime bsAb or isotypexCD40 bsAb (data from 1 representative experiment out of three).

Figure 26. Simultaneous binding of CD40 and CEA by CD40xCEA bsAbs mediates activation of tonsillar cancer APCs in vitro. Human CD45+ HLA-DR+ CD3- cells from a

tonsillar cancer biopsy were co-cultured with UV-irradiated CHO cells transfected with human CEA in the presence of CD40xCEA bsAb, CD40 mAb or isotype control. After 13 h culture, cells were harvested and the frequencies of CD86+ CD40+ cells were investigated using flow cytometry of CD19+ CD20+ B cells, CD14+ macrophages, CD1c+ cDC2s and XCR1+ cDC1s.

Figure 27. Accumulation of the CD40xCEA bsAb, but not corresponding CD40 mAb, in CEA-expressing tumors. Human CD40 transgenic mice were inoculated with MC38-hCEA tumor cells (MC-38-CEA-2, Kerafast) s.c. and were administered with 100 µg anti-CD40 antibody or a molar equivalent dose (167 µg) CD40xCEA bsAb or Isotype bsAb i.p. on days 10 and 13. On day 14, tumors were dissected. Frozen tumor sections were stained for human IgG to assess accumulation of administered antibodies, and for CEA to assess CEA expression pattern in the tumors. Representative images of (A) IgG staining and (B) CEA staining are shown. The staining pattern obtained following treatment with CD40xCEA is significantly stronger than for the controls, and the staining pattern is consistent with the CEA-staining of the tumor.

Figure 28. Cryo preserved tumors (B16.F10-hCD40+ # 6, 7 and 9 used as control, hereafter called B16 AND MB49 #2, 4 and 5) from human CD40 transgenic mice were analyzed. 8µm cryosections were prepared and stained. Mouse spleen was used as positive control. The sections were analyzed in a Leica DMRX-e microscope and representative photos were taken. A) representative images from MB49 tumors. B) The immunohistochemical staining was judged as follows; negative (0), few positive cells (1+), moderate numbers of positive cells (2+), high numbers of positive cells (3+) or very high numbers of positive cells (4+). The analysis shows that a marked higher degree of infiltrating T cells are seen in the MB49 tumors compared to the B16 tumors used as control. Antibodies used for staining: CD4: Rat IgG, Affymetrix,14-0042 1:200, CD8 Rat IgG, Affymetrix,14-0081 1:200, CD3 Rabbit, Dako, A0452 1:100, CD45 Rat IgG, Biodesign 1:100. C) Immune cell population frequencies in MB49-EpCAM tumors, with similar in vivo growth and immune infiltration as MB49 tumors. Human CD40 transgenic mice were injected with MB49-hEpCAM cells (0,25x106) s.c. into the right flank in 100 ul of PBS. 12 days after tumor inoculation, tumors were dissected, dissociated and stained for flow cytometry analysis of immune cell content. The frequencies of NK cells (CD45+, CD11b-, CD19- MHC II-, TCRbeta-, NK1.1+), T cells (CD45+, CD11b-, CD19- MHC II-, TCRbeta+, NK1.1-), B cells (CD45+, Ly6G-, CD3-, NK1.1-, CD19+), monocytes/macrophages (CD45+, Ly6G-, CD3-, NK1.1-, CD64+) and DCs (CD45+, Ly6G-, CD3-, NK1.1-, CD64-, CD11c+, MHC II+) within the total viable CD45+ population was assessed.

Figure 29. Data from NHP study. A) B cell activation of the cynoCEAxCD40 RUBY™ on cynomolgus and human B cells in the presence of CEA transfected cells (macaque CEA, NP_001040590.1). Primary cynomolgus B cells were cultured with titrated antibodies in the presence CEA expressed on CHO cells. After 2 days, expression of CD86 on B cells was analyzed by FACS. The graphs show pooled data from two cynomolgus and four human donors. The data demonstrate that CEAxCD40 bsAbs in the RUBY™ format induce upregulation of CD86 on cynomolgus and human B cells to a similar degree. The CEA-conditional activation of CD40 on cynomolgus B cells and human B cells is similar to what is observed with the human CEAxCD40 bsAb in RUBY ™ used for the in vitro assays. The cynoCEAxCD40 bsAb binds with similar affinity to human and cynomolgus monkey CEA (hCEA vs cCEA, right panel). In B) and C) key data from the toxicology assessment in cynomolgus monkey is presented. The cynoCEAxCD40 bispecific antibody was administered once weekly via intravenous infusion for 2 weeks to cynomolgus monkeys at two different dose levels (10 mg/kg and 37.5 mg/kg). One female and one male were evaluated at each dose level. B) Data on L-aspartate aminotransferase (ASAT) and L-alanine aminotransferase (ALAT) and C) levels of IL-6 and TNFalpha over time. In addition, plasma levels for the following cytokines were measured by a bead-based multiplex immunoassay: IL-2, IL-6, IL-8, IL-10, MCP-1, IFN-γ and TNF-α. The conclusion from the study was that there were no findings associated with cyoCEAxCD40 bsAb at the evaluated dose levels.

Figure 30. Structure of RUBY™ bsAb and binding of RUBY™ bsAb to their various antigen targets as measured by ELISA. (A) Chain 1 consists of the IgG heavy chain, a short polypeptide linker and the light chain of the additional Fab fragment, chain 2 is a light chain that binds to the VH and CH1 domains of the IgG part and chain 3 is a short heavy chain that binds to the light chain appended to the IgG. (B) Dual ELISA showing simultaneous binding of CD40xEpCAM RUBY™ bsAb to its respective antigen targets. ELISA plates were coated with human CD40, bsAb was added followed by detection using biotinylated EpCAM.(C) Dual ELISA showing simultaneous binding of CD40xCEA RUBY™ bsAb to its respective antigen targets. ELISA plates were coated with human CEACAM5, bsAb was added followed by detection using biotinylated CD40. (D) Mono ELISA showing binding of GFPxEpCAM control RUBY™ bsAb to human EpCAM. ELISA plates were coated with human EpCAM, bsAb was added followed by detection using goat anti human-kappa light chain-HRP. (E) Mono ELISA showing binding of cynoCEAxCD40 RUBY™ bsAb to human CD40. ELISA plates were coated with human CD40 followed by addition of cynoCEAxCD40 RUBY™ bsAb and detection using goat anti human-kappa light chain-HRP. (F). Mono ELISA showing binding of cynoCEAxCD40

RUBY™ bsAb to human CEACAM5. ELISA plates were coated with human CEA followed by addition of cynoCEAxCD40 RUBY™ bsAb and detection using goat anti human-kappa light chain-HRP. In summary, Bispecific antibodies were successfully generated in the RUBY™format and the generated bsAbs displayed good binding to their respective antigen targets as illustrated by the ELISA binding evaluations.

Figure 31. MC38-CEACAM5 2 tumor growth and survival. hCD40tg mice inoculated with MC38-CEACAM5 2 tumors were dosed with the indicated treatments on days 7, 10, and 13 post-inoculation. Tumors were frequently measured, and the graphs shows the mean tumor volume (+SD) of each group until the first mouse in any of the treatment groups reached a tumor volume above the ethical limit, and the % surviving mice in each treatment group.

Figure 32. Dissociated cells from human gastric cancer tumors were analyzed for: (left) their CEA-expression (gated on total viable cells), (middle) ability to provide cross-linking to CD40xCEA Neo-X-Prime bsAb (ffAC_05337) in a CD40 reporter assay, and (right, 1nM CD40xCEA) CD83 upregulation following stimulation of the tumor infiltrating immune cells (gated on viable CD45+CD3-CD56- cells) using CD40xCEA Neo-X-Prime bsAb or isotypexCD40 bsAb (data from 1 representative experiment out of four).

Figure 33. Effect of the bispecific antibody ffAC_05337on CD40 reporter cells when co-cultured with tumor cells with different CEA receptor density in the presence and absence of soluble CEA. MKN45, CEA high expressing cells (A), LS174T, CEA intermediate expressing cells (B), HT29 and LOVO, CEA low expressing cells (C-D). The response was calculated as fold induction to background.

Figure 34. Effect of the bispecific antibody ffAC_05337 on CD40 reporter cells co-cultured with CEA expressing CHO cells and titrated antibodies in the presence or absence of soluble CEA. The response was calculated as fold induction to background.

Sequence Tables

Table A – Binding domain B1 VL and VH amino acid (aa) and nucleotide (nt) sequences

SEQ ID NO.	ANTIBODY REF	TYPE	SEQUENCE

1	1132, light chain VL (also known as 1133)	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQYGRNPPTFGQGTKLEIK
2	1132, light chain VL (also known as 1133)	nt	gatattcagatgacccagagcccgagcagcctgagcgcgagcg tgggcgatcgcgtgaccattacctgccgcgcgagccagagcatt agcagctatctgaactggtatcagcagaaaccgggcaaagcgc cgaaactgctgatttatgcggcgagcagcctgcagagcggcgtg ccgagccgctttagcggcagcggcagcggcaccgattttaccct gaccattagcagcctgcagccggaagattttgcgacctattattg ccagcagtatggccgcaacccgccgacctttggccagggcacca aactggaaattaaa
3	1132, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSGIGSYGGGTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYVNFGMD YWGQGTLVTVSS
4	1132, heavy chain VH	nt	gaagtgcagctgctggaaagcggcggcggcctggtgcagccg ggcggcagcctgcgcctgagctgcgcggcgagcggctttacctt tagcagctatgcgatgagctgggtgcgccaggcgccgggcaaa ggcctggaatgggtgagcggcattggcagctatggcggcggca cctattatgcggatagcgtgaaaggccgctttaccattagccgcg ataacagcaaaaacaccctgtatctgcagatgaacagcctgcgc gcggaagataccgcggtgtattattgcgcgcgctatgtgaacttt ggcatggattattggggccagggcaccctggtgaccgtgagca gc
5	1150, light chain VL (also known as 1151)	aa	DIQMTQSPSSLSASVGDHVTITCRASQSISSYLNW YQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQYGSAPPTFGQGTKLEIK
6	1150, light chain VL (also known as 1151)	nt	gatattcagatgacccagagcccgagcagcctgagcgcgagcg tgggcgatcatgtgaccattacctgccgcgcgagccagagcatt agcagctatctgaactggtatcagcagaaaccgggcaaagcgc cgaaactgctgatttatgcggcgagcagcctgcagagcggcgtg ccgagccgctttagcggcagcggcagcggcaccgattttaccct gaccattagcagcctgcagccggaagattttgcgacctattattg

			ccagcagtatggcagcgcgccgccgacctttggccagggcacc aaactggaaattaaa
7	1150, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSGIGGSSSYTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYYSYHMD YWGQGTLVTVSS
8	1150, heavy chain VH	nt	gaagtgcagctgctggaaagcggcggcggcctggtgcagccg ggcggcagcctgcgcctgagctgcgcggcgagcggctttacctt tagcagctatgcgatgagctgggtgcgccaggcgccgggcaaa ggcctggaatgggtgagcggcattggcggcagcagcagctata ccagctatgcggatagcgtgaaaggccgctttaccattagccgc gataacagcaaaaacaccctgtatctgcagatgaacagcctgcg cgcggaagataccgcggtgtattattgcgcgcgctattatagcta tcatatggattattggggccagggcaccctggtgaccgtgagca gc
9	1140, light chain VL (also known as 1135)	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQSYSTPYTFGQGTKLEIK
10	1140, light chain VL (also known as 1135)	nt	gatattcagatgacccagagcccgagcagcctgagcgcgagcg tgggcgatcgcgtgaccattacctgccgcgcgagccagagcatt agcagctatctgaactggtatcagcagaaaccgggcaaagcgc cgaaactgctgatttatgcggcgagcagcctgcagagcggcgtg ccgagccgctttagcggcagcggcagcggcaccgattttaccct gaccattagcagcctgcagccggaagattttgcgacctattattg ccagcagagctatagcaccccgtatacctttggccagggcacca aactggaaattaaa
11	1140, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARGPVYSSV FDYWGQGTLVTVSS
12	1140, heavy chain VH	nt	gaagtgcagctgctggaaagcggcggcggcctggtgcagccg ggcggcagcctgcgcctgagctgcgcggcgagcggctttacctt tagcagctatgcgatgagctgggtgcgccaggcgccgggcaaa ggcctggaatgggtgagcgcgattagcggcagcggcggcagc acctattatgcggatagcgtgaaaggccgctttaccattagccgc

			gataacagcaaaaacaccctgtatctgcagatgaacagcctgcg cgcggaagataccgcggtgtattattgcgcgcgcggcccggtgt atagcagcgtgtttgattattggggccagggcaccctggtgacc gtgagcagc
13	1107, light chain VL (also known as 1108)	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQYGVYPFTFGQGTKLEIK
14	1107, light chain VL (also known as 1108)	nt	gatattcagatgacccagagcccgagcagcctgagcgcgagcg tgggcgatcgcgtgaccattacctgccgcgcgagccagagcatt agcagctatctgaactggtatcagcagaaaccgggcaaagcgc cgaaactgctgatttatgcggcgagcagcctgcagagcggcgtg ccgagccgctttagcggcagcggcagcggcaccgattttaccct gaccattagcagcctgcagccggaagattttgcgacctattattg ccagcagtatggcgtgtatccgtttacctttggccagggcaccaa actggaaattaaa
15	1107, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARRVWGFDY WGQGTLVTVSS
16	1107, heavy chain VH	nt	gaagtgcagctgctggaaagcggcggcggcctggtgcagccg ggcggcagcctgcgcctgagctgcgcggcgagcggctttacctt tagcagctatgcgatgagctgggtgcgccaggcgccgggcaaa ggcctggaatgggtgagcgcgattagcggcagcggcggcagc acctattatgcggatagcgtgaaaggccgctttaccattagccgc gataacagcaaaaacaccctgtatctgcagatgaacagcctgcg cgcggaagataccgcggtgtattattgcgcgcgccgcgtgtggg gctttgattattggggccagggcaccctggtgaccgtgagcagc
17	G12, light chain VL	aa	QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVY WYQQLPGTAPKLLIYGNINRPSGVPDRFSGSKSGTS ASLAISGLRSEDEADYYCAAWDKSISGLVFGGGTKL TVLG
18	G12, light chain VL	nt	cagagcgtgctgacccagccgccgagcgcgagcggcaccccg ggccagcgcgtgaccattagctgcaccggcagcagcagcaaca ttggcgcgggctataacgtgtattggtatcagcagctgccgggc accgcgccgaaactgctgatttatggcaacattaaccgcccgag cggcgtgccggatcgctttagcggcagcaaaagcggcaccagc gcgagcctggcgattagcggcctgcgcagcgaagatgaagcg

			gattattattgcgcggcgtgggataaaagcattagcggcctggt gtttggcggcggcaccaaactgaccgtgctgggg
19	G12, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMH WVRQAPGKGLEWLSYISGGSSYIFYADSVRGRFTIS RDNSENALYLQMNSLRAEDTAVYYCARILRGGSGM DLWGQGTLVTVSS
20	G12, heavy chain VH	nt	gaagtgcagctgctggaaagcggcggcggcctggtgcagccg ggcggcagcctgcgcctgagctgcgcggcgagcggctttacctt tagcacctatggcatgcattgggtgcgccaggcgccgggcaaa ggcctggaatggctgagctatattagcggcggcagcagctatat tttttatgcggatagcgtgcgcggccgctttaccattagccgcgat aacagcgaaaacgcgctgtatctgcagatgaacagcctgcgcg cggaagataccgcggtgtattattgcgcgcgcattctgcgcggc ggcagcggcatggatctgtggggccagggcaccctggtgaccg tgagcagc
21	APX005, light chain VL	aa	DIQMTQSPSSLSASVGDRVTIKCQASQSISSRLAW YQQKPGKPPKLLIYRASTLASGVPSRFSGSGSGTDF TLTISSLQPEDVATYYCQCTGYGISWPIGGGTKVEIK
22	APX005, light chain VL	nt	gatattcagatgacccagagcccgagcagcctgagcgcgagcg tgggcgatcgcgtgaccattaaatgccaggcgagccagagcatt agcagccgcctggcgtggtatcagcagaaaccgggcaaaccgc cgaaactgctgatttatcgcgcgagcaccctggcgagcggcgtg ccgagccgctttagcggcagcggcagcggcaccgattttaccct gaccattagcagcctgcagccggaagatgtggcgacctattatt gccagtgcaccggctatggcattagctggccgattggcggcggc accaaagtggaaattaaa
23	APX005, heavy chain VH	aa	QVQLVESGGGVVQPGRSLRLSCAASGFSFSSTYVC WVRQAPGKGLEWIACIYTGDGTNYSASWAKGRFTI SKDSSKNTVYLQMNSLRAEDTAVYFCARPDITYGFA INFWGPGTLVTVSS
24	APX005, heavy chain VH	nt	caggtgcagctggtggaaagcggcggcggcgtggtgcagccg ggccgcagcctgcgcctgagctgcgcggcgagcggctttagctt tagcagcacctatgtgtgctgggtgcgccaggcgccgggcaaa ggcctggaatggattgcgtgcatttataccggcgatggcaccaa ctatagcgcgagctgggcgaaaggccgctttaccattagcaaag atagcagcaaaaacaccgtgtatctgcagatgaacagcctgcgc gcggaagataccgcggtgtatttttgcgcgcgcccggatattacc

			tatggctttgcgattaacttttggggcccgggcaccctggtgacc gtgagcagc
25	21.4.1, light chain VL	aa	DIQMTQSPSSVSASVGDRVTITCRASQGIYSWLAW YQQKPGKAPNLLIYTASTLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQANIFPLTFGGGTKVEIK
26	21.4.1, light chain VL	nt	gatattcagatgacccagagcccgagcagcgtgagcgcgagcg tgggcgatcgcgtgaccattacctgccgcgcgagccagggcatt tatagctggctggcgtggtatcagcagaaaccgggcaaagcgc cgaacctgctgatttataccgcgagcaccctgcagagcggcgtg ccgagccgctttagcggcagcggcagcggcaccgattttaccct gaccattagcagcctgcagccggaagattttgcgacctattattg ccagcaggcgaacatttttccgctgacctttggcggcggcaccaa agtggaaattaaa
27	21.4.1, heavy chain VH	aa	QVQLVQSGAEVKKPGASVKVSCKASGYTFTGYYMH WVRQAPGQGLEWMGWINPDSGGTNYAQKFQGRV TMTRDTSISTAYMELNRLRSDDTAVYYCARDQPLGY CTNGVCSYFDYWGQGTLVTVSS
28	21.4.1, heavy chain VH	nt	caggtgcagctggtgcagagcggcgcggaagtgaaaaaaccg ggcgcgagcgtgaaagtgagctgcaaagcgagcggctatacct ttaccggctattatatgcattgggtgcgccaggcgccgggccag ggcctggaatggatgggctggattaacccggatagcggcggca ccaactatgcgcagaaatttcagggccgcgtgaccatgacccgc gataccagcattagcaccgcgtatatggaactgaaccgcctgcg cagcgatgataccgcggtgtattattgcgcgcgcgatcagccgct gggctattgcaccaacggcgtgtgcagctattttgattattgggg ccagggcaccctggtgaccgtgagcagc
17	G12_mut, light chain VL	aa	QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVY WYQQLPGTAPKLLIYGNINRPSGVPDRFSGSKSGTS ASLAISGLRSEDEADYYCAAWDKSISGLVFGGGTKL TVL
18	G12_mut, light chain VL	nt	cagagcgtgctgacccagccgccgagcgcgagcggcaccccg ggccagcgcgtgaccattagctgcaccggcagcagcagcaaca ttggcgcgggctataacgtgtattggtatcagcagctgccgggc accgcgccgaaactgctgatttatggcaacattaaccgcccgag cggcgtgccggatcgctttagcggcagcaaaagcggcaccagc gcgagcctggcgattagcggcctgcgcagcgaagatgaagcg

			gattattattgcgcggcgtgggataaaagcattagcggcctggt gtttggcggcggcaccaaactgaccgtgctgggg
29	G12_mut, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMH WVRQAPGKGLEWLSYISGGSSYIFYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARILRGGSGM DLWGQGTLVTVSS
30	G12_mut, heavy chain VH	nt	gaagtgcagctgctggaaagcggcggcggcctggtgcagccg ggcggcagcctgcgcctgagctgcgcggcgagcggctttacctt tagcacctatggcatgcattgggtgcgccaggcgccgggcaaa ggcctggaatggctgagctatattagcggcggcagcagctatat tttttatgcggatagcgtgaagggccgctttaccattagccgcgat aacagcaaaaacacgctgtatctgcagatgaacagcctgcgcg cggaagataccgcggtgtattattgcgcgcgcattctgcgcggc ggcagcggcatggatctgtggggccagggcaccctggtgaccg tgagcagc
430	ffAC_05337 light chain VL CD40 binder	aa	QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVY WYQRLPGTAPKLLIYGNINRPSGVPDRFSGSKSGTS ASLAISGLRSEDEADYYCAAWDKSISGLVFGGGTKL TVL
434	ffAC_05337 light chain VL CD40 binder	nt	cagagcgtgctgacccagccgccgagcgcgagcggcaccccg ggccagcgcgtgaccattagctgcaccggcagcagcagcaaca ttggcgcgggctataacgtgtattggtatcagcggctgccgggc accgcgccgaaactgctgatttatggcaacattaaccgcccgag cggcgtgccggatcgctttagcggcagcaaaagcggcaccagc gcgagcctggcgattagcggcctgcgcagcgaagatgaagcg gattattattgcgcggcgtgggataaaagcattagcggcctggt gtttggcggcggcaccaaactgaccgtgctgggg
431	ffAC_05337 heavy chain VH CD40 binder	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMH WVREAPGKGLEWLSYISGGSSYIFYADSVKGRFTIS RDNSKNTLYLQMNSLRAEDTAVYYCARILRGGSGM DLWGQGTLVTVSS
435	ffAC_05337 heavy chain VH CD40 binder	nt	gaagtgcagctgctggaaagcggcggcggcctggtgcagccg ggcggcagcctgcgcctgagctgcgcggcgagcggctttacctt tagcacctatggcatgcattgggtgcgcgaggcgccgggcaaa ggcctggaatggctgagctatattagcggcggcagcagctatat tttttatgcggatagcgtgaagggccgctttaccattagccgcgat aacagcaaaaacacgctgtatctgcagatgaacagcctgcgcg

			cggaagataccgcggtgtattattgcgcgcgcattctgcgcggc ggcagcggcatggatctgtggggccagggcaccctggtgaccg tgagcagc

Table B – Binding domain B2 VL and VH amino acid (aa) and nucleotide (nt) sequences

SEQ ID NO.	ANTIBODY REF	TYPE	SEQUENCE
31	AC_05059, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
32	AC_05059, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
33	AC_05059, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPP HLDYWGQGTLVTVSS
34	AC_05059, heavy chain VH	nt	gaggtgcagctgttggagagcgggggaggcttggtacagcctg gggggtccctgcgcctctcctgtgcagccagcggattcacctttc ttcttcttacatgggttgggtccgccaggctccagggaaggggct ggagtgggtctcatctattggttctggttcttactctacatcttatgc agactccgtgaagggccggttcaccatctcccgtgacaattccaa gaacacgctgtatctgcaaatgaacagcctgcgtgccgaggaca cggctgtatattattgtgcgcgctacccgtctgttccgttcccgcct catttggactattggggccagggaaccctggtcaccgtctcctca
35	AC_05060, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSIRDYLNW YQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQGTFPFTFGQGTKLEIK

36	AC_05060, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagtctattag ggactatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag ggtactttcccgttcacttttggccaggggaccaagctggagatc aaa
37	AC_05060, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYYMS WVRQAPGKGLEWVSGISGYGYYTGYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARHGYGVID YWGQGTLVTVSS
38	AC_05060, heavy chain VH	nt	gaggtgcagctgttggagagcgggggaggcttggtacagcctg gggggtccctgcgcctctcctgtgcagccagcggattcacctttg gttcttactacatgtcttgggtccgccaggctccagggaaggggc tggagtgggtctcaggtatttctggttacggttactacacaggtta tgcagactccgtgaagggccggttcaccatctcccgtgacaattc caagaacacgctgtatctgcaaatgaacagcctgcgtgccgagg acacggctgtatattattgtgcgcgccatggttacggtgttattga ctattggggccagggaaccctggtcaccgtctcctca
39	AC_05061, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQGAYVPYTFGQGTKLEIK
40	AC_05061, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag ggtgcttacgttccgtacacttttggccaggggaccaagctggag atcaaa
41	AC_05061, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYGYTHFD YWGQGTLVTVSS
42	AC_05061, heavy chain VH	nt	gaggtgcagctgttggagagcgggggaggcttggtacagcctg gggggtccctgcgcctctcctgtgcagccagcggattcaccttta gcagctatgccatgagctgggtccgccaggctccagggaaggg

			gctggagtgggtctcagctattagtggtagtggtggtagcacata ctatgcagactccgtgaagggccggttcaccatctcccgtgacaa ttccaagaacacgctgtatctgcaaatgaacagcctgcgtgccg aggacacggctgtatattattgtgcgcgctacggttacactcattt tgactattggggccagggaaccctggtcaccgtctcctca
43	AC_05062, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQAISGYLNW YQQKPGKAPKLLIYSASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQSYSTPYTFGQGTKLEIK
44	AC_05062, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcaggctattag cggttatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctattctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag agttacagtacccccttatacttttggccaggggaccaagctggag atcaaa
45	AC_05062, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYRWHGSV FDYWGQGTLVTVSS
46	AC_05062, heavy chain VH	nt	gaggtgcagctgttggagagcgggggaggcttggtacagcctg gggggtccctgcgcctctcctgtgcagccagcggattcaccttta gcagctatgccatgagctgggtccgccaggctccagggaaggg gctggagtgggtctcagctattagtggtagtggtggtagcacata ctatgcagactccgtgaagggccggttcaccatctcccgtgacaa ttccaagaacacgctgtatctgcaaatgaacagcctgcgtgccg aggacacggctgtatattattgtgcgcgctaccgttggcatggttc tgtttttgactattggggccagggaaccctggtcaccgtctcctca
47	AC_05064, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQYPWYFPYTFGQGTKLEIK
48	AC_05064, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag

			tacccgtggtacttcccgtacacttttggccaggggaccaagctg gagatcaaa
49	AC_05064, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYGYSVLD YWGQGTLVTVSS
50	AC_05064, heavy chain VH	nt	gaggtgcagctgttggagagcgggggaggcttggtacagcctg gggggtccctgcgcctctcctgtgcagccagcggattcaccttta gcagctatgccatgagctgggtccgccaggctccagggaaggg gctggagtgggtctcagctattagtggtagtggtggtagcacata ctatgcagactccgtgaagggccggttcaccatctcccgtgacaa ttccaagaacacgctgtatctgcaaatgaacagcctgcgtgccg aggacacggctgtatattattgtgcgcgctacggttactctgttttg gactattggggccagggaaccctggtcaccgtctcctca
51	AC_05079, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
52	AC_05079, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
53	AC_05079, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPP PLDYWGQGTLVTVSS
54	AC_05079, heavy chain VH	nt	gaggtacagctgcttgagtctggaggtggactggtacagcccgg ggggtccctgaggctctcctgtgctgcctccggtttcacctttagc agctcttatatggggtgggtcaggcaggctcctggtaagggcct cgagtgggtgtccagcatcggaagcggatcatacagcacgagt tacgccgactcagtaaagggtagattcaccatttcacgcgacaa cagcaagaacacattgtatctccaaatgaattctctgagagcgg aagacacagcagtgtactattgcgccagatatccttccgtgccctt tcctccacccccttgattactggggacagggtactcttgtgactgtct cctca

55	AC_05080, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
56	AC_05080, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
57	AC_05080, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPP LLDYWGQGTLVTVSS
58	AC_05080, heavy chain VH	nt	gaggttcagttgctggagtcagggggcggattggtgcagcctgg tggtagtctccgtcttagctgcgcggcttcagggttcacttttagca gctcatacatgggctgggtgcggcaggcaccaggaaagggcct ggaatgggtgagtagtataggatctggcagctatagtacttcata tgctgatagtgtgaaaggacgatttactatctctcgtgacaattca aaaaacacccttacttgcagatgaatagccttagggcggagga taccgcggtttactattgtgctcgttatccgagcgtgccttccccc ccctttggactactggggacaaggcaccctcgtgacagtctcct ca
59	AC_05081, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
60	AC_05081, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
61	AC_05081, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI

			SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFQP HLDYWGQGTLVTVSS
62	AC_05081, heavy chain VH	nt	gaagtacagctgctggaaagcggtggaggactcgtgcagcctg gtgggtccctcaggctctcctgtgcagcgagcggttttacattctc tagttcatatatggggtgggtacggcaggccccaggtaagggct tagagtgggtaagcagtattggatccgggtcatacagtacatcct atgccgactccgtcaagggtaggttcacgatcagccgggataac tcaaagaatactctctacctccaaatgaattcactgcgggccgag gatacagcagtttactattgtgcaagatatccatccgtgccctttc agccccacctggactactggggtcagggaaccctggtaacagtc tcctca
63	AC_05082, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
64	AC_05082, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
65	AC_05082, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYHPYSFDY WGQGTLVTVSS
66	AC_05082, heavy chain VH	nt	gaggtgcagctgttggagagcgggggaggcttggtacagcctg gggggtccctgcgcctctcctgtgcagccagcggattcaccttta gcagctatgccatgagctgggtccgccaggctccagggaaggg gctggagtgggtctcagctattagtggtagtggtggtagcacata ctatgcagactccgtgaagggccggttcaccatctcccgtgacaa ttccaagaacacgctgtatctgcaaatgaacagcctgcgtgccg aggacacggctgtatattattgtgcgcgctaccacccgtactctt tgactattggggccagggaaccctggtcaccgtctcctca
67	AC_05083, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSIRGYLNW YQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQPSYPSLFTFGQGTKLEIK

68	AC_05083, light chain VL	nt	gatattcagatgacgcagagccctagttctctgtctgcttccgttg gggaccgtgtaaccatcacgtgtagggctagtcagtccatacgc ggatatttgaactggtatcagcagaaaccagggaaagctccaa agttgctcatttatgcagcatcaagcttacagagcggcgtgccca gccgtttcagcgggtcaggaagcgggacggacttcacgttgacc atatcttctctgcagcccgaggatttcgcgacctactattgtcagc aaccaagctacccgtctctgttcactttcggccaaggaacgaagc ttgaaatcaag
69	AC_05083, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYSPYVLDY WGQGTLVTVSS
70	AC_05083, heavy chain VH	nt	gaggtgcaactgctggagagcggcggaggcctggtccagcca ggcgggtctctcagactgagttgcgccgccagcggctttacttttt cctcttatgctatgagctgggtacgacaggcgcccggaaaaggc ctggaatgggtttccgccatctctggctccggcggttctacctact acgctgattccgtcaagggcaggtttaccatcagcagggacaat agcaagaacacactgtacctccagatgaactctttgcgcgcaga ggacacagccgtttactattgcgccaggtacagcccatacgtgct cgactactggggccagggtacactcgtgacggtctcctca
71	AC_05084, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQVDGLFTFGQGTKLEIK
72	AC_05084, light chain VL	nt	gatattcagatgactcagagcccctcatccctgtccgctagcgtg ggggaccgagtgactattacatgcagagcctctcagtccatatca tcctatctgaattggtaccagcaaaagcccggaaaagcaccaaa actgctcatttatgccgctagttcacttcagtctggggttccgtctc ggtttagcggatctggcagcggtacagactttacacttaccatca gcagtctgcagccagaggactttgcgacgtactattgtcaacaa gtcgacggcttattcacctttggacagggcaccaagttggagatt aaa
106	AC_05084, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARVYYPAVM DYWGQGTLVTVSS
107	AC_05084, heavy chain VH	nt	gaagtacagctgttggagtctggaggtggattggttcagcccgg ggggagccttaggctgagttgtgcagcttcaggatttactttcagt tcctacgctatgtcatgggtcagacaggcgccagggaagggact

			ggaatgggtgtctgctatcagcggaagtggagggtctacttact acgcagactctgttaagggccggtttaccatctcccgagataaca gcaagaatactttatacctgcagatgaactcccttcgcgccgaag acactgctgtctactattgcgctcgggtatattatcctgccgtcatg gactattggggccagggaaccctcgtcactgtctcctca
108	AC_05085, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
109	AC_05085, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
110	AC_05085, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYYMY WVRQAPGKGLEWVSSIGGYSGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARNTPFPGG SGLDYWGQGTLVTVSS
111	AC_05085, heavy chain VH	nt	gaggtgcagctgttggagagcgggggaggcttggtacagcctg gggggtccctgcgcctctcctgtgcagccagcggattcacctttg gttcttactacatgtactgggtccgccaggctccagggaagggg ctggagtgggtctcatctattggtggttactctggttctacatacta tgcagactccgtgaagggccggttcaccatctcccgtgacaattc caagaacacgctgtatctgcaaatgaacagcctgcgtgccgagg acacggctgtatattattgtgcgcgcaacactccgttcccgggtg gttctggtttggactattggggccagggaaccctggtcaccgtct cctca
112	AC_05086, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
113	AC_05086, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag

			gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
114	AC_05086, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAHYPSVPFPP HLDYWGQGTLVTVSS
115	AC_05086, heavy chain VH	nt	gaggtccagctgcttgaatccggaggcggcctggtccaaccagg cggaagtctccgcttatcatgcgccgcatccggctttacgttcagt tcatcatatatggggtgggtccggcaggcgccaggtaagggcct tgaatgggtctcctcaattggctcaggatcctattccaccagctat gctgattccgtgaagggccgctttacaatcagtcgcgacaattct aagaacaccctgtacctgcagatgaactctctgagagcagaag atacagccgtttattattgtgcacactatccttccgtgccattccca cctcatctggattactggggccaggggacgctggtcactgtctcc tca
116	AC_05087, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
117	AC_05087, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
118	AC_05087, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPPVPFPP HLDYWGQGTLVTVSS
119	AC_05087, heavy chain VH	nt	gaggtgcagcttctggagagtgggggcgggctcgtgcagcctg gggggtccctccgtctcagttgtgcagcttcaggctttacctttagt agttcatacatgggatgggtccgtcaggctcctgggaagggctt agaatgggtgtcatcaattggctccggctcctattctacatcctac gccgacagtgttaagggtcgttttaccattagcagggataacagt aagaatacattgtacctccaaatgaattctctgcgggcggaagat actgccgtgtactattgcgcaagatacccacctgtcccgttccctc

			cgcaccttgattactgggggcagggtactctggtgaccgtctcct ca
120	AC_05088, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
121	AC_05088, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
122	AC_05088, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVLFPP HLDYWGQGTLVTVSS
123	AC_05088, heavy chain VH	nt	gaagtgcagcttctggagtctggtggaggtctggtgcagcctgg agggtctctgagacttagttgtgcagcatctggttttaccttcagct caagctacatgggctgggtgagacaggcacccggaaaaggatt agagtgggtgagctccatcgggtctggcagctactctacctccta cgctgactctgttaagggacgattcaccatttccagagacaatag caaaaacacactgtacttacaaatgaattctctccgtgctgagga tacagcggtctactattgtgctcgatacccgtctgttcttttccccc tcaccttgattattgggggcagggcacgctggtgacagtctcctc a
124	AC_05089, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
125	AC_05089, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa

126	AC_05089, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPH HLDYWGQGTLVTVSS
127	AC_05089, heavy chain VH	nt	gaagtccagttgttagagagtgggggcgggctggtgcagccag ggggttctcttaggttgtcatgtgccgcctccggcttcactttctctt cttcctacatgggctgggtgcggcaggcaccgggaaagggtctg gagtgggtgtctagtattggctccggctcctacagtacttcatacg cagattcagtgaaagggaggttcaccatctcaagagataacagc aaaaacaccctgtacttgcagatgaattccctgcgggccgaaga taccgccgtctactactgcgcacggtacccctccgttcccttccccc accatctggactactggggtcaaggcactttggtcacagtctcctc a
128	AC_05090, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
129	AC_05090, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
130	AC_05090, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPL HLDYWGQGTLVTVSS
131	AC_05090, heavy chain VH	nt	gaggtgcagctgttggagtcagggggaggcttggtgcagcccg gaggctccctgcgcctgtcatgcgcagcctctgggtttacattctc tagctcttatatgggctgggtgaggcaagctcctggcaagggac tcgagtgggtctcttccatcggctccggtagctacagtacgagtta tgcagacagtgtgaaaggtagatttactatctccagggacaactc caagaataccctctacctgcagatgaattccctcagagccgaag atactgcagtgtactattgcgccaggtacccctccgtcccattccc cctccaccttgattactggggacagggaaccctggtaactgtctc ctca

132	AC_05091, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
133	AC_05091, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
134	AC_05091, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPP HFDYWGQGTLVTVSS
135	AC_05091, heavy chain VH	nt	gaagtacaattgttagagagcggaggggactcgttcagcccg gaggatcactgcgcctgtcatgtgcagctagcggtttcacttttag ttcatcctacatgggttgggtcagacaggccccagggaaaggcc ttgagtgggtgtcctccattgggtctggtagctactcaacatcata cgctgacagcgtcaagggacgattcaccattagtcgcgacaact ctaagaatacactctacctccagatgaactctctcagggccgagg acacagccgtgtattactgtgcacgctatccctctgtacccttcct ccacattttgactattggggtcaggggaccttggtcactgtctcct ca
136	AC_05092, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSDLNW YQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
137	AC_05092, light chain VL	nt	gatattcagatgacacagtcccccagtagtctgagcgcctcagtt ggtgacagagtgacaataacctgtagggcttctcagagcatatc cagcgatctgaactggtatcagcagaaaccagggaaggccccc aaattgctcatctatgccgcatccagccttcagagcggagtgcctt cacggttcagtggttcagggtcaggaacagacttcacgctcacg atcagttctctgcaacccgaagatttcgcaacttactactgtcaac aggccggcaaccctcataccttcggtcagggaacgaaattgga gatcaag
138	AC_05092, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI

			SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPP HLDYWGQGTLVTVSS
139	AC_05092, heavy chain VH	nt	gaggtgcagctgttagaaagtgggggaggccttgtccaaccag gaggtagtctgcgcctcagttgcgccgcgtctggctttactttctct tcaagctatatggggtgggtgcgacaggctccaggcaagggac tggaatgggtgtcttcaattggttcaggttcctactcaacaagcta tgcggattcagtgaagggtagatttacgatcagtagggacaata gcaagaacaccctctacctccagatgaactcacttagagccgag gatacagccgtgtactattgtgctaggtatccatccgtgcccttcc cccctcaccttgactactggggccaaggtacactcgtgaccgtct cctca
140	AC_05093, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
141	AC_05093, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
142	AC_05093, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPP HVDYWGQGTLVTVSS
143	AC_05093, heavy chain VH	nt	gaggttcaacttttagagagtggtggtgggctggtgcagcctgg cgggagcctccgcctctcatgcgcagccagtgggtttacctttag ctccagttacatgggctgggtgagacaggcccctggaaaaggg ctggaatgggtgtctagcatcggcagcggctcatattctacgtctt acgctgacagcgttaaaggcaggtttaccatctccagggacaat tcaaagaacactctgtatcttcagatgaacagtctcagagctgag gacaccgctgtgtattattgcgcccgataccttccgtgccattcc caccccacgtagactactggggccaggggaccctcgtcacggtc tcctca
144	AC_05094, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK

145	AC_05094, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
146	AC_05094, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPP HWDYWGQGTLVTVSS
147	AC_05094, heavy chain VH	nt	gaagtacagctgctggagagtggtggtggtctggtgcagcccg ggggctccctgcggctttcctgtgccgcgtctggcttcaccttcag ctcatcttacatgggctgggttcgacaggcacctgggaagggttt agagtgggtgtctagcattgggagtgggtcctattcaacatccta cgcagatagtgtgaagggccggtttaccatctctagagacaaca gcaagaataccttatacttacaaatgaatagcctgagagcagag gataccgctgtctattattgtgcacggtaccctagcgtcccgtttcc ccctcactgggactattggggccaggggactctggtgaccgtctc ctca
148	AC_05095, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
149	AC_05095, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
150	AC_05095, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPS HLDYWGQGTLVTVSS
151	AC_05095, heavy chain VH	nt	gaggtgcagctgttggaatctggaggaggcctcgtgcagccag gaggttccctgaggctgtcttgcgccgcctcaggtttcacctttag

			ctcttcctacatgggatgggtgcggcaagcacccggaaaaggg ctggagtgggtgagctccatcggctcaggttcttatagcacttctt atgcggactccgttaaaggccgctttactatcagcagggacaact ccaagaatacactgtatctgcagatgaacagcctgcgtgctgaa gacaccgcagtctattactgcgcaagatatccgtccgttccatttc caagccacctggattactggggccaggggacactggtgaccgtc tcctca
152	AC_05096, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
153	AC_05096, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
154	AC_05096, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFRP HLDYWGQGTLVTVSS
155	AC_05096, heavy chain VH	nt	gaggtgcagctgctggagtcagggggaggccttgttcaaccgg gaggcagtctgagattatcatgtgcggcttcagggtttaccttctc cagtagttatatgggctgggtccgccaggctccaggtaaggggt tggaatgggtgtcttctatcggctctggatcctattctacgtcctac gccgattctgtcaaaggaaggttcaccatctccagggataattct aagaataccctctacctgcaaatgaactccctgcgagccgaaga tacagccgtttactactgcgcgagatacccgagcgtgcctttcag gccccatctggattactggggacaggggacacttgtgacagtct cctca
156	AC_05097, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
157	AC_05097, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc

			acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
158	AC_05097, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRQAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFSP HLDYWGQGTLVTVSS
159	AC_05097, heavy chain VH	nt	gaagtgcagctccttgagtccggtgggggcctcgtccagcccgg cggatccctgaggctgtcatgcgctgcaagcggcttcacatttag cagcagttatatgggctgggttagacaggctccgggcaaggga ctggaatgggtcagcagtattggtagcgggtcatatagtacttca tacgccgatagtgtgaagggccggttcacaatttccagggataa ctccaaaaatacactgtatctgcaaatgaactctctgcgagcgga agacactgctgtttactactgtgccaggtatccgagtgtgccctttt ctccacacctggactattggggccaaggaacccttgtgaccgtct cctca
160	AC_05098, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSIRDYLNW YQQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDF TLTISSLQPEDFATYYCQQGTFPFTFGQGTKLEIK
161	AC_05098, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagtctattag ggactatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag ggtactttcccgttcacttttggccaggggaccaagctggagatc aaa
162	AC_05098, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYYMS WVRQAPGKGLEWVSGISGYGYYTGYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARNGYGVID YWGQGTLVTVSS
163	AC_05098, heavy chain VH	nt	gaggtccagctcctggaatcaggtggtgggctcgtacagccagg aggttcacttcggctgtcttgcgcagccagcgggttcacatttggc tcttactacatgtcttgggtcaggcaggcccctggcaagggttta gagtgggtcagtggaatatctggttatgggtactacacaggttat gcggacagcgtcaagggcaggtttaccatatctagagacaata gtaagaacacccttatttgcagatgaactctctgagagctgaag

			acacagccgtttattattgcgcccggaacgggtatggagtgattg attattgggggcagggtactctggttacagtctcctca
164	AC_05099, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
165	AC_05099, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
166	AC_05099, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYGYTHFD YWGQGTLVTVSS
167	AC_05099, heavy chain VH	nt	gaggtgcagctgttggagagcgggggaggcttggtacagcctg gggggtccctgcgcctctcctgtgcagccagcggattcaccttta gcagctatgccatgagctgggtccgccaggctccagggaaggg gctggagtgggtctcagctattagtggtagtggtggtagcacata ctatgcagactccgtgaagggccggttcaccatctcccgtgacaa ttccaagaacacgctgtatctgcaaatgaacagcctgcgtgccg aggacacggctgtatattattgtgcgcgctacggttacactcattt tgactattggggccagggaaccctggtcaccgtctcctca
168	AC_05100, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
169	AC_05100, light chain VL	nt	gacatccagatgacccagtctccatcctccctgagcgcatctgta ggagaccgcgtcaccatcacttgccgggcaagtcagagcattag cagctatttaaattggtatcagcagaaaccagggaaagccccta agctcctgatctatgctgcatccagtttgcaaagtggggtcccatc acgtttcagtggcagtggaagcgggacagatttcactctcaccat cagcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
170	AC_05100, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMS WVRQAPGKGLEWVSAISGSGGSTYYADSVKGRFTI

			SRDNSKNTLYLQMNSLRAEDTAVYYCARYWWSSYY GYLDYWGQGTLVTVSS
171	AC_05100, heavy chain VH	nt	gaagttcaactcctcgaatctggtgggggtctggtccagcccgg gggcagccttaggctcagttgcgctgccagcggtttcacattctct agctacgccatgagttgggtgcggcaggcaccaggaaagggat tggaatgggtcagtgcaatctcaggcagtggcggctccacttact atgctgattccgttaaggggcgattcaccatcagtcgtgataattc taaaaatacactgtatctgcagatgaattctttgcgcgctgagga cacagctgtgtattattgcgcccggtattggtggtccagctattac gggtatctggactattggggtcaggggactcttgttacagtctcct ca
172	Fab1, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQSSHGPLLTFGQGTKLEIK
173	Fab1, light chain VL	nt	gacatccagatgacccagtctccatcctccctgtctgcatctgtag gagacagagtcaccatcacttgccgggcaagtcagagcattagc agctatttaaattggtatcagcagaaaccagggaaagcccctaa gctcctgatctatgctgcatccagtttgcaaagtggggtcccatca aggttcagtggcagtggatctgggacagatttcactctcaccatc agcagtctgcaacctgaagattttgcaacttactactgtcaacagt cctcacacggccctttgctgacttttggccaggggaccaagctgg agatcaaa
174	Fab1, heavy chain VH	aa	QVQLVQSGAEVKKPGSSVKVSCKASGGTFGYYAIH WVRQAPGQGLEWMGGIGSIFGTANYAQKFQGRVT ITADESTSTAYMELSSLRSEDTAVYYCARAWSSDH MDYWGQGTLVTVSS
175	Fab1, heavy chain VH	nt	caggttcagctggttcagagcggtgcagaagttaaaaaaccgg gtagcagcgttaaagttagctgtaaagcaagcggtggcaccttt ggctattatgcaattcactgggttcgtcaggcacctggtcaaggt ctggaatggatgggtggtattggttcgatttttggcaccgcaaatt atgcccagaaatttcagggtcgtgttaccattaccgcagatgaaa gcaccagcaccgcatatatggaactgagcagcctgcgtagcga agataccgcagtgtattattgtgcacgtgcatggagttcggatca tatggactactggggccagggaaccctggtcaccgtctcctca
176	Fab2, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWRSHLFTFGQGTKLEIK

177	Fab2, light chain VL	nt	gacatccagatgacccagtctccatcctccctgtctgcatctgtag gagacagagtcaccatcacttgccgggcaagtcagagcattagc agctatttaaattggtatcagcagaaaccagggaaagcccctaa gctcctgatctatgctgcatccagtttgcaaagtggggtcccatca aggttcagtggcagtggatctgggacagatttcactctcaccatc agcagtctgcaacctgaagattttgcaacttactactgtcaacagt ggcgctcacacctttttacttttggccaggggaccaagctggaga tcaaa
178	Fab2, heavy chain VH	aa	QVQLVQSGAEVKKPGSSVKVSCKASGGTFHDGAIS WVRQAPGQGLEWMGHIIPIDGTAGYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARYRFYGIDY WGQGTLVTVSS
179	Fab2, heavy chain VH	nt	caggttcagctggttcagagcggtgcagaagttaaaaaaccgg gtagcagcgttaaagttagctgtaaagcaagcggtggcaccttt cacgatggtgcaattagctgggttcgtcaggcacctggtcaaggt ctggaatggatgggtcacattattccgattgatggcaccgcagg atatgcccagaaatttcagggtcgtgttaccattaccgcagatga aagcaccagcaccgcatatatggaactgagcagcctgcgtagc gaagataccgcagtgtattattgtgcacgttaccgtttctatggaa tcgactactggggccagggaaccctggtcaccgtctcctca
180	Fab3, light chain VL	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAW YQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQQYWYPLTFGQGTKLEIK
181	Fab3, light chain VL	nt	gaaattgttctgacccagagtccgggtacactgagcctgtcaccg ggtgaacgtgcaaccctgagctgtcgtgcaagccagagcgttag cagcagctatctggcatggtatcagcagaaacctggtcaggcac cgcgtctgctgatttatggtgcaagcagccgtgcaaccggtattc cggatcgttttagcggtagcggtagtggcaccgattttaccctga ccattagccgtctggaaccggaagattttgcagtgtattattgtca gcagtattggtaccctctgacttttggccaggggaccaagctgga gatcaaa
182	Fab3, heavy chain VH	aa	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSSIH WVRQAPGQGLEWMGHIYPSFGTANYAQKFQGRVT ITADESTSTAYMELSSLRSEDTAVYYCARHSGSRFF SPMDYWGQGTLVTVSS
183	Fab3, heavy chain VH	nt	caggttcagctggttcagagcggtgcagaagttaaaaaaccgg gtagcagcgttaaagttagctgtaaagcaagcggtggcaccttt agcagcagcagtattcactgggttcgtcaggcacctggtcaagg

			tctggaatggatgggtcatatttacccgtcttttggcaccgcaaat tatgcccagaaatttcagggtcgtgttaccattaccgcagatgaa agcaccagcaccgcatatatggaactgagcagcctgcgtagcg aagataccgcagtgtattattgtgcacgtcacagcggatctcgct tttttagtccgatggactactggggccagggaaccctggtcaccg tctcctca
184	Fab4, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQPWTYLFTFGQGTKLEIK
185	Fab4, light chain VL	nt	gacatccagatgacccagtctccatcctccctgtctgcatctgtag gagacagagtcaccatcacttgccgggcaagtcagagcattagc agctatttaaattggtatcagcagaaaccagggaaagcccctaa gctcctgatctatgctgcatccagtttgcaaagtggggtcccatca aggttcagtggcagtggatctgggacagatttcactctcaccatc agcagtctgcaacctgaagattttgcaacttactactgtcaacag ccatggacctacttgtttacttttggccaggggaccaagctggag atcaaa
186	Fab4, heavy chain VH	aa	QVQLVQSGAEVKKPGSSVKVSCKASGGTFDDHAIS WVRQAPGQGLEWMGGIIPIFSYAYYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARGRFYFPPS LDYWGQGTLVTVSS
187	Fab4, heavy chain VH	nt	caggttcagctggttcagagcggtgcagaagttaaaaaaccgg gtagcagcgttaaagttagctgtaaagcaagcggtggcaccttt gacgatcacgcaattagctgggttcgtcaggcacctggtcaagg tctggaatggatgggtggtattattccgatttttagctacgcatatt atgcccagaaatttcagggtcgtgttaccattaccgcagatgaaa gcaccagcaccgcatatatggaactgagcagcctgcgtagcga agataccgcagtgtattattgtgcacgtgggcgtttctactttcccc cgtccctcgactactggggccagggaaccctggtcaccgtctcct ca
188	Fab5, light chain VL	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAW YQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQQPAAYLPTFGQGTKLEIK
189	Fab5, light chain VL	nt	gaaattgttctgacccagagtccgggtacactgagcctgtcaccg ggtgaacgtgcaaccctgagctgtcgtgcaagccagagcgttag cagcagctatctggcatggtatcagcagaaacctggtcaggcac cgcgtctgctgatttatggtgcaagcagccgtgcaaccggtattc cggatcgttttagcggtagcggtagtggcaccgattttaccctga

			ccattagccgtctggaaccggaagattttgcagtgtattattgtca gcagcctgcagcttaccttccaacttttggccaggggaccaagct ggagatcaaa
190	Fab5, heavy chain VH	aa	QVQLVQSGAEVKKPGSSVKVSCKASGGTFGSDAIG WVRQAPGQGLEWMGGIIPHFDTAYYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARTYYTYAFF DYWGQGTLVTVSS
191	Fab5, heavy chain VH	nt	caggttcagctggttcagagcggtgcagaagttaaaaaaccgg gtagcagcgttaaagttagctgtaaagcaagcggtggcaccttt ggaagcgatgcaattgggtgggttcgtcaggcacctggtcaag gtctggaatggatgggtggtattattccgcattttgataccgcata ttatgcccagaaatttcagggtcgtgttaccattaccgcagatga aagcaccagcaccgcatatatggaactgagcagcctgcgtagc gaagataccgcagtgtattattgtgcacgtacttattacacgtatg ccttctttgactactggggccagggaaccctggtcaccgtctcctc a
192	Fab6, light chain VL	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAW YQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQQHVYGAPYTFGQGTKLEIK
193	Fab6, light chain VL	nt	gaaattgttctgacccagagtccgggtacactgagcctgtcaccg ggtgaacgtgcaaccctgagctgtcgtgcaagccagagcgttag cagcagctatctggcatggtatcagcagaaacctggtcaggcac cgcgtctgctgatttatggtgcaagcagccgtgcaaccggtattc cggatcgttttagcggtagcggtagtggcaccgattttaccctga ccattagccgtctggaaccggaagattttgcagtgtattattgtca gcagcatgtgtatggagctccatacacttttggccaggggacca agctggagatcaaa
194	Fab6, heavy chain VH	aa	QVQLVQSGAEVKKPGSSVKVSCKASGGTFSGGYIS WVRQAPGQGLEWMGGIIPYFHHANYAQKFQGRVTI TADESTSTAYMELSSLRSEDTAVYYCARGVWRLDY WGQGTLVTVSS
195	Fab6, heavy chain VH	nt	caggttcagctggttcagagcggtgcagaagttaaaaaaccgg gtagcagcgttaaagttagctgtaaagcaagcggtggcaccttt agcggtggctacattagctgggttcgtcaggcacctggtcaagg tctggaatggatgggtggtattattccgtattttcatcatgcaaatt atgcccagaaatttcagggtcgtgttaccattaccgcagatgaaa gcaccagcaccgcatatatggaactgagcagcctgcgtagcga

			agataccgcagtgtattattgtgcacgtggcgtgtggcgtctcga ctactggggccagggaaccctggtcaccgtctcctca
196	Fab7, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQWGYLLTFGQGTKLEIK
197	Fab7, light chain VL	nt	gacatccagatgacccagtctccatcctccctgtctgcatctgtag gagacagagtcaccatcacttgccgggcaagtcagagcattagc agctatttaaattggtatcagcagaaaccagggaaagcccctaa gctcctgatctatgctgcatccagtttgcaaagtggggtcccatca aggttcagtggcagtggatctgggacagatttcactctcaccatc agcagtctgcaacctgaagattttgcaacttactactgtcaacagt ggggatacctgttgacttttggccaggggaccaagctggagatc aaa
198	Fab7, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSDHMY WVRQAPGKGLEWVSAIYGSHGSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPRYGSID YWGQGTLVTVSS
199	Fab7, heavy chain VH	nt	gaggtgcagctgttggagtctgggggaggcttggtacagcctgg ggggtccctgagactctcctgtgcagcctctggattcacctttagc agcgatcatatgtattgggtccgccaggctccagggaaggggct ggagtgggtctcagctatttacggtagtcatggtagcacaagcta cgcagactccgtgaagggccggttcaccatctccagagacaatt ccaagaacacgctgtatctgcaaatgaacagcctgagagccga ggacacggccgtatattactgtgcgcgctatccgcggtacggatc tattgactactggggccagggaaccctggtcaccgtctcctca
200	Fab8, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQSYSGPPTFGQGTKLEIK
201	Fab8, light chain VL	nt	gacatccagatgacccagtctccatcctccctgtctgcatctgtag gagacagagtcaccatcacttgccgggcaagtcagagcattagc agctatttaaattggtatcagcagaaaccagggaaagcccctaa gctcctgatctatgctgcatccagtttgcaaagtggggtcccatca aggttcagtggcagtggatctgggacagatttcactctcaccatc agcagtctgcaacctgaagattttgcaacttactactgtcaacagt catattcaggacctccgacttttggccaggggaccaagctggag atcaaa
202	Fab8, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFGDHAMS WVRQAPGKGLEWVSAISGYGHSTGYADSVKGRFTI

			SRDNSKNTLYLQMNSLRAEDTAVYYCARNHYRVGL DYWGQGTLVTVSS
203	Fab8, heavy chain VH	nt	gaggtgcagctgttggagtctgggggaggcttggtacagcctgg ggggtccctgagactctcctgtgcagcctctggattcacctttggc gaccatgccatgagctgggtccgccaggctccagggaaggggc tggagtgggtctcagctattagtggttacggtcatagcacaggct acgcagactccgtgaagggccggttcaccatctccagagacaat tccaagaacacgctgtatctgcaaatgaacagcctgagagccga ggacacggccgtatattactgtgcgcgcaatcattaccgcgtagg cctggactactggggccagggaaccctggtcaccgtctcctca
204	Fab9, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQNSSSRLLTFGQGTKLEIK
205	Fab9, light chain VL	nt	gacatccagatgacccagtctccatcctccctgtctgcatctgtag gagacagagtcaccatcacttgccgggcaagtcagagcattagc agctatttaaattggtatcagcagaaaccagggaaagcccctaa gctcctgatctatgctgcatccagtttgcaaagtggggtcccatca aggttcagtggcagtggatctgggacagatttcactctcaccatc agcagtctgcaacctgaagattttgcaacttactactgtcaacag aattcatctagccggcttttgacttttggccaggggaccaagctg gagatcaaa
206	Fab9, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFGDYDMS WVRQAPGKGLEWVSGIGHSGGSTYYADSVKGRFT ISRDNSKNTLYLQMNSLRAEDTAVYYCARASDWYP SGFDYWGQGTLVTVSS
207	Fab9, heavy chain VH	nt	gaggtgcagctgttggagtctgggggaggcttggtacagcctgg ggggtccctgagactctcctgtgcagcctctggattcacctttggc gactatgatatgagctgggtccgccaggctccagggaaggggc tggagtgggtctcagggattggtcatagtggtggtagcacatact acgcagactccgtgaagggccggttcaccatctccagagacaat tccaagaacacgctgtatctgcaaatgaacagcctgagagccga ggacacggccgtatattactgtgcgcgcgcctccgattggtaccc atctggattcgactactggggccagggaaccctggtcaccgtctc ctca
208	Fab10, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QQKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQSFSHPPTFGQGTKLEIK

209	Fab10, light chain VL	nt	gacatccagatgacccagtctccatcctccctgtctgcatctgtag gagacagagtcaccatcacttgccgggcaagtcagagcattagc agctatttaaattggtatcagcagaaaccagggaaagcccctaa gctcctgatctatgctgcatccagtttgcaaagtggggtcccatca aggttcagtggcagtggatctgggacagatttcactctcaccatc agcagtctgcaacctgaagattttgcaacttactactgtcaacag agcttttctcacccaccgacttttggccaggggaccaagctggag atcaaa
210	Fab10, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFYDHAMS WVRQAPGKGLEWVSAISGSYGSTGYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARWGGWAG DIDYWGQGTLVTVSS
211	Fab10, heavy chain VH	nt	gaggtgcagctgttggagtctgggggaggcttggtacagcctgg ggggtccctgagactctcctgtgcagcctctggattcacctttac gaccatgccatgagctgggtccgccaggctccagggaaggggc tggagtgggtctcagctattagtggtagttacggtagcacaggat acgcagactccgtgaagggccggttcaccatctccagagacaat tccaagaacacgctgtatctgcaaatgaacagcctgagagccga ggacacggccgtatattactgtgcgcgctggggtggatgggccg gagacatcgactactggggccagggaaccctggtcaccgtctcc tca
212	Fab11, light chain VL	aa	EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAW YQQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDF TLTISRLEPEDFAVYYCQQRDWFPLFTFGQGTKLEIK
213	Fab11, light chain VL	nt	gaaattgttctgacccagagtccgggtacactgagcctgtcaccg ggtgaacgtgcaaccctgagctgtcgtgcaagccagagcgttag cagcagctatctggcatggtatcagcagaaacctggtcaggcac cgcgtctgctgatttatggtgcaagcagccgtgcaaccggtattc cggatcgttttagcggtagcggtagtggcaccgattttaccctga ccattagccgtctggaaccggaagattttgcagtgtattattgtca gcagcgtgactggtttcctttatttacttttggccaggggaccaag ctggagatcaaa
214	Fab11, heavy chain VH	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDHAMH WVRQAPGKGLEWVSAISGYGGYTHYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYGGYSGD FDYWGQGTLVTVSS
215	Fab11, heavy chain VH	nt	gaggtgcagctgttggagtctgggggaggcttggtacagcctgg ggggtccctgagactctcctgtgcagcctctggattcacctttagc

			gatcacgccatgcattgggtccgccaggctccagggaaggggct ggagtgggtctcagctattagtggttatggtggttatacacactac gcagactccgtgaagggccggttcaccatctccagagacaattc caagaacacgctgtatctgcaaatgaacagcctgagagccgag gacacggccgtatattactgtgcgcgctatggcggatatagtgg ggattttgactactggggccagggaaccctggtcaccgtctcctc a
384	Mab2, light chain VL	aa	DIQMTQSPSSLSASVGDRVTITCRASENIFSYLAWY QQKPGKAPKLLIYNTRTLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQHHYGTPFTFGQGTKLEIK
385	Mab2, light chain VL	nt	gacatccagatgacacagagccctagcagcctgtctgccagcgt gggagacagagtgaccatcacctgtagagccagcgagaacatc ttcagctacctggcctggtatcagcagaagcctggcaaggcccct aagctgctgatctacaacacccggacactgcagagcggcgtgcc aagcagattttctggcagcggctctggcaccgacttcaccctgac catatctagcctgcagcctgaggacttcgccacctactactgcca gcaccactacggcacccctttcacatttggccagggcaccaagct ggaaatcaag
386	Mab2, heavy chain VH	aa	EVQLVESGGGLVQPGGSLRLSCAASGFVFSSYDMS WVRQAPGKGLEWVSYISSGGGITYYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCAAHYFGSSG PFAYWGQGTLVTVSS
387	Mab2, heavy chain VH	nt	gaggtgcagctggttgaatctggcggaggactggttcagcctgg cggatctctgagactgtcttgtgccgccagcggcttcgtgttcagc agctacgatatgagctgggtccgacaggcccctggcaaaggac ttgagtgggtgtcctacatcagcagcggcggaggcatcacctac tacgccgattctgtgaagggcagattcaccatcagccgggacaa cagcaagaacaccctgtacctgcagatgaacagcctgagagcc gaggacaccgccgtgtactattgtgccgctcactacttcggcagc tctggccctttgcctattggggccagggcacactggtcaccgtta gctct
432	ffAC_05337 light chain VL CEA binder	aa	DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWY QEKPGKAPKLLIYAASSLQSGVPSRFSGSGSGTDFT LTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIK
436	ffAC_05337 light chain VL CEA binder	nt	acatccagatgacccagtctccatcctccctgagcgcatctgtag gagaccgcgtcaccatcacttgccgggcaagtcagagcattagc agctatttaaattggtatcaggagaaaccagggaaagcccctaa gctcctgatctatgctgcatccagtttgcaaagtggggtcccatca

			cgtttcagtggcagtggaagcgggacagatttcactctcaccatc agcagtctgcaacctgaagattttgcaacttattactgtcaacag gctggtaacccgcacacttttggccaggggaccaagctggagat caaa
433	ffAC_05337 heavy chain VH CEA binder	aa	EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMG WVRRAPGKGLEWVSSIGSGSYSTSYADSVKGRFTI SRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPL HLDYWGQGTLVTVSS
437	ffAC_05337 heavy chain VH CEA binder	nt	gaggtgcagctgttggagtcagggggaggcttggtgcagcccg gaggctccctgcgcctgtcatgcgcagcctctgggtttacattctc tagctcttatatgggctgggtgaggcgagctcctggcaagggac tcgagtgggtctcttccatcggctccggtagctacagtacgagtta tgcagacagtgtgaaaggtagatttactatctccagggacaactc caagaataccctctacctgcagatgaattccctcagagccgaag atactgcagtgtactattgcgccaggtacccctccgtcccattccc cctccaccttgattactggggacagggaaccctggtaactgtctc ctca

Table C(1) – Exemplary heavy chain CDR sequences (binding domain B1)

Antibody ref (VH)	**SEQ**	**H CDR1**	**SEQ**	**H CDR2**	**SEQ**	**H CDR3**
1132	73	GFTFSSYA	74	IGSYGGGT	75	ARYVNFGMDY
1150	73	GFTFSSYA	76	IGGSSSYT	77	ARYYSYHMDY
1140	73	GFTFSSYA	78	ISGSGGST	79	ARGPVYSSVFDY
1107	73	GFTFSSYA	78	ISGSGGST	80	ARRVWGFDY
G12 and G12_mut	81	GFTFSTYG	82	ISGGSSYI	83	ARILRGGSGMDL
APX005	84	GFSFSSTY	85	IYTGDGTN	86	ARPDITYGFAINF
21.4.1	87	GYTFTGYY	88	INPDSGGT	89	ARDQPLGYCTNGVCSYFDY

Table C(2) – Exemplary light chain CDR sequences (binding domain B1)

Antibody ref (VL)	**SEQ**	**L CDR1**	**SEQ**	**L CDR2**	**SEQ**	**L CDR3**
1132 (1133)	90	QSISSY	91	AAS	92	QQYGRNPPT
1150 (1151)	90	QSISSY	91	AAS	93	QQYGSAPPT
1140 (1135)	90	QSISSY	91	AAS	94	QQSYSTPYT
1107 (1108)	90	QSISSY	91	AAS	95	QQYGVYPFT

G12 and G12_mut	96	SSNIGAGYN	97	GNI	98	AAWDKSISGLV
APX005	99	QSISSR	100	RAS	101	QCTGYGISWP
21.4.1	102	QGIYSW	103	TAS	104	QQANIFPLT

Table D(1a) – Exemplary heavy chain CDR sequences (binding domain B2)

Antibody ref (VH)	**SEQ**	**H CDR1**	**SEQ**	**H CDR2**	**SEQ**	**H CDR3**
AC_05059	216	GFTFSSSY	217	IGSGSYST	218	ARYPSVPFPPHLDY
AC_05060	219	GFTFGSYY	220	ISGYGYYT	221	ARHGYGVIDY
AC_05061	222	GFTFSSYA	223	ISGSGGST	224	ARYGYTHFDY
AC_05062	222	GFTFSSYA	223	ISGSGGST	225	ARYRWHGSVFDY
AC_05064	222	GFTFSSYA	223	ISGSGGST	226	ARYGYSVLDY
AC_05079	216	GFTFSSSY	217	IGSGSYST	227	ARYPSVPFPPPLDY
AC_05080	216	GFTFSSSY	217	IGSGSYST	228	ARYPSVPFPPLLDY
AC_05081	216	GFTFSSSY	217	IGSGSYST	229	ARYPSVPFQPHLDY
AC_05082	222	GFTFSSYA	223	ISGSGGST	230	ARYHPYSFDY
AC_05083	222	GFTFSSYA	223	ISGSGGST	231	ARYSPYVLDY
AC_05084	222	GFTFSSYA	223	ISGSGGST	232	ARVYYPAVMDY
AC_05085	219	GFTFGSYY	233	IGGYSGST	234	ARNTPFPGGSGLDY
AC_05086	216	GFTFSSSY	217	IGSGSYST	235	AHYPSVPFPPHLDY
AC_05087	216	GFTFSSSY	217	IGSGSYST	236	ARYPPVPFPPHLDY
AC_05088	216	GFTFSSSY	217	IGSGSYST	237	ARYPSVLFPPHLDY
AC_05089	216	GFTFSSSY	217	IGSGSYST	238	ARYPSVPFPHHLDY
AC_05090	216	GFTFSSSY	217	IGSGSYST	239	ARYPSVPFPLHLDY
AC_05091	216	GFTFSSSY	217	IGSGSYST	240	ARYPSVPFPPHFDY
AC_05092	216	GFTFSSSY	217	IGSGSYST	218	ARYPSVPFPPHLDY
AC_05093	216	GFTFSSSY	217	IGSGSYST	241	ARYPSVPFPPHVDY
AC_05094	216	GFTFSSSY	217	IGSGSYST	242	ARYPSVPFPPHWDY
AC_05095	216	GFTFSSSY	217	IGSGSYST	243	ARYPSVPFPSHLDY
AC_05096	216	GFTFSSSY	217	IGSGSYST	244	ARYPSVPFRPHLDY
AC_05097	216	GFTFSSSY	217	IGSGSYST	245	ARYPSVPFSPHLDY
AC_05098	219	GFTFGSYY	220	ISGYGYYT	246	ARNGYGVIDY
AC_05099	222	GFTFSSYA	223	ISGSGGST	224	ARYGYTHFDY
AC_05100	222	GFTFSSYA	223	ISGSGGST	247	ARYWWSSYYGYLDY
Fab1	248	GGTFGYYA	249	IGSIFGTA	250	ARAWSSDHMDY
Fab2	251	GGTFHDGA	252	IIPIDGTA	253	ARYRFYGIDY

Fab3	254	GGTFSSSS	255	IYPSFGTA	256	ARHSGSRFFSPMDY
Fab4	257	GGTFDDHA	258	IIPIFSYA	259	ARGRFYFPPSLDY
Fab5	260	GGTFGSDA	261	IIPHFDTA	262	ARTYYTYAFFDY
Fab6	263	GGTFSGGY	264	IIPYFHHA	265	ARGVWRLDY
Fab7	266	GFTFSSDH	267	IYGSHGST	268	ARYPRYGSIDY
Fab8	269	GFTFGDHA	270	ISGYGHST	271	ARNHYRVGLDY
Fab9	272	GFTFGDYD	335	IGHSGGST	273	ARASDWYPSGFDY
Fab10	274	GFTFYDHA	275	ISGSYGST	276	ARWGGWAGDIDY
Fab11	277	GFTFSDHA	278	ISGYGGYT	279	ARYGGYSGDFDY

Table D(1b) – Exemplary heavy chain CDR extended sequences (binding domain B2)

* Ext refers to extended sequences – these are sequence variants of the CDRs with additional flanking amino acids; they are functionally comparable to the unextended CDR sequences in Table D1a.

Antibody ref (VH)	**SEQ**	**Ext H CDR1***	**SEQ**	**Ext H CDR2***
AC_05059	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05060	282	GFTFGSYYMS	283	GISGYGYYTG
AC_05061	284	GFTFSSYAMS	285	AISGSGGSTY
AC_05062	284	GFTFSSYAMS	285	AISGSGGSTY
AC_05064	284	GFTFSSYAMS	285	AISGSGGSTY
AC_05079	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05080	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05081	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05082	284	GFTFSSYAMS	285	AISGSGGSTY
AC_05083	284	GFTFSSYAMS	285	AISGSGGSTY
AC_05084	284	GFTFSSYAMS	285	AISGSGGSTY
AC_05085	286	GFTFGSYYMY	287	SIGGYSGSTY
AC_05086	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05087	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05088	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05089	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05090	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05091	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05092	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05093	280	GFTFSSSYMG	281	SIGSGSYSTS

AC_05094	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05095	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05096	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05097	280	GFTFSSSYMG	281	SIGSGSYSTS
AC_05098	282	GFTFGSYYMS	283	GISGYGYYTG
AC_05099	288	GFTFSSYAMS	285	AISGSGGSTY
AC_05100	288	GFTFSSYAMS	285	AISGSGGSTY
Fab1	289	GGTFGYYAIH	290	GIGSIFGTAN
Fab2	291	GGTFHDGAIS	292	HIIPIDGTAG
Fab3	293	GGTFSSSSIH	294	HIYPSFGTAN
Fab4	295	GGTFDDHAIS	296	GIIPIFSYAY
Fab5	297	GGTFGSDAIG	298	GIIPHFDTAY
Fab6	299	GGTFSGGYIS	300	GIIPYFHHAN
Fab7	301	GFTFSSDHMY	302	AIYGSHGSTS
Fab8	303	GFTFGDHAMS	304	AISGYGHSTG
Fab9	305	GFTFGDYDMS	306	GIGHSGGSTY
Fab10	307	GFTFYDHAMS	308	AISGSYGSTG
Fab11	309	GFTFSDHAMH	310	AISGYGGYTH

Table D(2) – Exemplary light chain CDR sequences (binding domain B2)

Antibody ref (VL)	**SEQ**	**L CDR1**	**SEQ**	**L CDR2**	**SEQ**	**L CDR3**
AC_05059	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05060	312	QSIRDY	91	AAS	313	QQGTFPFT
AC_05061	90	QSISSY	91	AAS	314	QQGAYVPYT
AC_05062	315	QAISGY	316	SAS	94	QQSYSTPYT
AC_05064	90	QSISSY	91	AAS	317	QQYPWYFPYT
AC_05079	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05080	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05081	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05082	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05083	318	QSIRGY	91	AAS	319	QQPSYPSLFT
AC_05084	90	QSISSY	91	AAS	320	QQVDGLFT
AC_05085	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05086	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05087	90	QSISSY	91	AAS	311	QQAGNPHT

AC_05088	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05089	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05090	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05091	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05092	321	QSISSD	91	AAS	311	QQAGNPHT
AC_05093	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05094	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05095	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05096	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05097	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05098	312	QSIRDY	91	AAS	313	QQGTFPFT
AC_05099	90	QSISSY	91	AAS	311	QQAGNPHT
AC_05100	90	QSISSY	91	AAS	311	QQAGNPHT
Fab1	90	QSISSY	91	AAS	322	QQSSHGPLLT
Fab2	90	QSISSY	91	AAS	323	QQWRSHLFT
Fab3	324	QSVSSSY	325	GAS	326	QQYWYPLT
Fab4	90	QSISSY	91	AAS	327	QQPWTYLFT
Fab5	324	QSVSSSY	325	GAS	328	QQPAAYLPT
Fab6	324	QSVSSSY	325	GAS	329	QQHVYGAPYT
Fab7	90	QSISSY	91	AAS	330	QQWGYLLT
Fab8	90	QSISSY	91	AAS	331	QQSYSGPPT
Fab9	90	QSISSY	91	AAS	332	QQNSSSRLLT
Fab10	90	QSISSY	91	AAS	333	QQSFSHPPT
Fab11	324	QSVSSSY	325	GAS	334	QQRDWFPLFT

Control antibody sequences

3174 VH aa (SEQ ID NO: 422)

EVQLLEQSGAELVRPGTSVKISCKASGYAFTNYWLGWVKQRPGHGLEWIGDIFPGSGNIHYNE
KFKGKATLTADKSSSTAYMQLSSLTFEDSAVYFCARLRNWDEPMDYWGQGTTVTVSS

3174 VH nt (SEQ ID NO: 423)

GAGGTGCAGCTGCTGGAACAGTCTGGCGCCGAACTCGTTAGACCTGGCACAAGCGTGAAGA
TCAGCTGCAAGGCCAGCGGCTACGCCTTCACAAATTATTGGCTCGGCTGGGTCAAACAGAG
GCCAGGACACGGACTGGAATGGATCGGCGATATCTTCCCCGGCAGCGGCAACATCCACTAC
AACGAGAAGTTCAAGGGCAAAGCCACACTGACCGCCGACAAGAGCAGCAGCACAGCCTATA
TGCAGCTGAGCAGCCTGACCTTCGAGGACAGCGCCGTGTACTTCTGCGCCAGGCTGAGAAA
CTGGGACGAGCCTATGGATTACTGGGGCCAGGGCACCACAGTGACAGTGTCTAGC

3174 VL aa (SEQ ID NO: 424)
ELVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWASTRESG
VPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYSYPLTFGAGTKLEIK

3174 VL nt (SEQ ID NO: 425)
GAACTGGTTATGACACAGAGCCCTAGCAGCCTGACAGTGACAGCCGGCGAGAAAGTGACAA
TGAGCTGCAAGAGCAGCCAGAGCCTGCTGAACAGCGGCAACCAGAAGAACTACCTGACCTG
GTATCAGCAGAAGCCCGGACAGCCTCCTAAGCTGCTGATCTATTGGGCCAGCACCAGAGAA
AGCGGCGTGCCCGATAGATTCACAGGCAGCGGCAGCGGAACCGACTTTACCCTGACAATTA
GCAGCGTGCAGGCCGAGGACCTGGCCGTGTATTATTGTCAGAACGACTACAGCTACCCTCT
GACCTTCGGAGCCGGCACCAAGCTGGAAATCAAG

1210 VH aa (SEQ ID NO: 426)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYYGGYYSAWMDYWGQGTLVTVSS

1210 VH nt (SEQ ID NO: 427)
GAGGTGCAGCTGCTCGAGAGCGGGGGAGGCTTGGTACAGCCTGGGGGGTCCCTGCGCCTC
TCCTGTGCAGCCAGCGGATTCACCTTTAGCAGCTATGCCATGAGCTGGGTCCGCCAGGCTCC
AGGGAAGGGGCTGGAGTGGGTCTCAGCTATTAGTGGTAGTGGTGGTAGCACATACTATGCA
GACTCCGTGAAGGGCCGGTTCACCATCTCCCGTGACAATTCCAAGAACACGCTGTATCTGCA
AATGAACAGCCTGCGTGCCGAGGACACGGCTGTATATTATTGTGCGCGCTACTACGGTGGTT
ACTACTCTGCTTGGATGGACTATTGGGGCCAGGGAACCCTGGTCACCGTCTCCTCA

1210 VL aa (SEQ ID NO: 428)
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQTYGYLHTFGQGTKLEIK

1210 VL nt (SEQ ID NO: 429)
GACATCCAGATGACCCAGTCTCCATCCTCCCTGAGCGCATCTGTAGGAGACCGCGTCACCAT
CACTTGCCGGGCAAGTCAGAGCATTAGCAGCTATTTAAATTGGTATCAGCAGAAACCAGGGA
AAGCCCCTAAGCTCCTGATCTATGCTGCATCCAGTTTGCAAAGTGGGGTCCCATCACGTTTC
AGTGGCAGTGGAAGCGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTT
TGCAACTTATTACTGTCAACAGACTTACGGTTACCTGCACACTTTTGGCCAGGGGACCAAGCT
GGAGATCAAA

Mutated IgG1 antibody sequence

IgG1 LALA-sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK (SEQ ID NO: 336)

Linker sequences

SGGGGSGGGGS (SEQ ID NO: 337)

SGGGGSGGGGSAP (SEQ ID NO: 338)

NFSQP (SEQ ID NO: 339)

KRTVA (SEQ ID NO: 340)

GGGSGGGG (SEQ ID NO: 341)

GGGGSGGGGS (SEQ ID NO: 342)

GGGGSGGGGSGGGGS (SEQ ID NO: 343)

GSTSGSGKPGSGEGSTKG (SEQ ID NO: 344)

THTCPPCPEPKSSDK (SEQ ID NO: 345)

GGGS (SEQ ID NO: 346)

EAAKEAAKGGGGS (SEQ ID NO: 347)

EAAKEAAK (SEQ ID NO: 348)

(SG)m, where m = 1 to 7.

IgG constant region sequences

IgG1 heavy chain constant region sequence:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPK
PKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLH
QDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPS
DIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYT
QKSLSLSPGK

[SEQ ID NO: 349]

IgG1 light chain constant region sequence:

RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[SEQ ID NO: 350]

Modified IgG4 heavy chain constant region sequence:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNRYTQKSL
SLSLGK

[SEQ ID NO: 351]

Modified IgG4 heavy chain constant region sequence:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPPCPAPEFLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA
VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLGK

[SEQ ID NO: 352]

Wild type IgG4 heavy chain constant region sequence:

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSL
SSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEFLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKGLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIA

VEWESNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSL
SLSLGK
[SEQ ID NO: 353]

Reference sequence CH1 (SEQ ID NO: 354):
ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYS
LSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKV**EPKSC**
(wherein the bold and underlined section is part of the hinge region, but is present in the appended Fab fragment in RUBY bsAb)

Reference sequence CKappa (SEQ ID NO: 355):
RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD
STYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Reference sequence CLambda, Immunoglobulin constant lambda 1 (SEQ ID NO: 356)
GQPKANPTVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADGSPVKAGVETTKPSKQSNN
KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Reference sequence CLambda, Immunoglobulin constant lambda 2 (SEQ ID NO: 357)
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN
KYAASSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Reference sequence CLambda, Immunoglobulin constant lambda 3 (SEQ ID NO: 358)
GQPKAAPSVTLFPPSSEELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNN
KYAASSYLSLTPEQWKSHKSYSCQVTHEGSTVEKTVAPTECS

Exemplary sequences of CD40 x CEACAM5 RUBY™ bsAb

Multi34

Chain H1 (SEQ ID NO: 359)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMHWVRRAPGKGLEWLSYISGGSSYIFYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARILRGGSGMDLWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVATGPAVLQSSGLYSLSSVVTV
PSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMI
SRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGKGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQRKPGKAP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSSHGPLLTFGQGTKLEIKRP

VAAPAVFIFPPSDEQLKSGTASVVCLLKNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Chain L1 (SEQ ID NO: 360)
QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVYWYQELPGTAPKLLIYGNINRPSGVPDRF
SGSKSGTSASLAISGLRSEDEADYYCAAWDKSISGLVFGGGTKLTVLGQPKAAPSVTLFPPSSE
ELQANKATLVCYISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAAWSYLSLTPEQWK
SHRSYSCQVTHEGSTVEKTVAPTECS

Chain H2 (SEQ ID NO: 361)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFGYYAIHWVREAPGQGLEWMGGIGSIFGTANYAQ
KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAWSSDHMDYWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLTSVVEVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

Multi42
Chain H1 (SEQ ID NO: 362)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSSIHWVRRAPGQGLEWMGHIYPSFGTANYAQ
KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHSGSRFFSPMDYWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVATGPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGKGGGGSGGGGSGGGGSQSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVYWYQRL
PGTAPKLLIYGNINRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDKSISGLVFGGG
TKLTVLGQPKAAPAVTLFPPSSEELQANKATLVCLIKDFYPGAVTVAWKADSSPVKAGVETTTPS
KQSNNKYAAWSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Chain L1 (SEQ ID NO: 363)
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQEKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDFAVYYCQQYWYPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCYLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLWSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

Chain H2 (SEQ ID NO: 364)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMHWVREAPGKGLEWLSYISGGSSYIFYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARILRGGSGMDLWGQGTLVTVSSASTKGPS

VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLTSVVEVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

Multi46

Chain H1 (SEQ ID NO: 365)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRRAPGKGLEWVSGIGSYGGGTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYVNFGMDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVATGPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGKGGGGSGGGGSGGGGSDIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQRKPGKAP
KLLIYAASSLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQQSSHGPLLTFGQGTKLEIKRP
VAAPAVFIFPPSDEQLKSGTASVVCLLKNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST
YSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Chain L1 (SEQ ID NO: 372)
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQEKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYGRNPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCYLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLWSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

Chain H2 (SEQ ID NO: 366)
QVQLVQSGAEVKKPGSSVKVSCKASGGTFGYYAIHWVREAPGQGLEWMGGIGSIFGTANYAQ
KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAWSSDHMDYWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLTSVVEVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

ffAC_05337 also known as ffAC_5337, ffP_A_05337 or ffP_A_5337

Chain H1 (SEQ ID NO: 367)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRRAPGKGLEWVSSIGSGSYSTSYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPLHLDYWGQGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVATGPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS

LSPGKGGGGSGGGGSGGGGSQSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVYWYQRLP
GTAPKLLIYGNINRPSGVPDRFSGSKSGTSASLAISGLRSEDEADYYCAAWDKSISGLVFGGGT
KLTVLGQPKAAPAVTLFPPSSEELQANKATLVCLIKDFYPGAVTVAWKADSSPVKAGVETTTPSK
QSNNKYAAWSYLSLTPEQWKSHRSYSCQVTHEGSTVEKTVAPTECS

Chain L1 (SEQ ID NO: 368)
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQEKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCYLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLWSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

Chain H2 (SEQ ID NO: 369)
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMHWVREAPGKGLEWLSYISGGSSYIFYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARILRGGSGMDLWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLTSVVEVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSC

Exemplary full heavy and light chain sequences

Binding domain B1:

1132/1133 Heavy chain, including RUBY mutations (VH: Q44R, CH1: H168A, F170G, CH2: L234A, L235A) (SEQ ID NO: 371):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRRAPGKGLEWVSGIGSYGGGTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYVNFGMDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVATGPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

1132/1133 Light chain, including RUBY mutations (VL: Q44E, CKappa: L135Y, S176W) (SEQ ID NO: 372):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQEKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYGRNPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCYLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLWSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

1132/1133 Heavy chain (SEQ ID NO: 378):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGIGSYGGGTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYVNFGMDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

1132/1133 Light chain (SEQ ID NO: 379):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYGRNPPTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

G12 Heavy chain (SEQ ID NO: 380):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWLSYISGGSSYIFYADS
VRGRFTISRDNSENALYLQMNSLRAEDTAVYYCARILRGGSGMDLWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

G12 Light chain (SEQ ID NO: 381):
QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVYWYQQLPGTAPKLLIYGNINRPSGVPDRF
SGSKSGTSASLAISGLRSEDEADYYCAAWDKSISGLVFGGGTKLTVLGQPKAAPSVTLFPPSSE
ELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKS
HRSYSCQVTHEGSTVEKTVAPTECS

G12_mut Heavy chain (SEQ ID NO: 382):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSTYGMHWVRQAPGKGLEWLSYISGGSSYIFYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARILRGGSGMDLWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG

KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

G12_mut Light chain (SEQ ID NO: 383):
QSVLTQPPSASGTPGQRVTISCTGSSSNIGAGYNVYWYQQLPGTAPKLLIYGNINRPSGVPDRF
SGSKSGTSASLAISGLRSEDEADYYCAAWDKSISGLVFGGGTKLTVLGQPKAAPSVTLFPPSSE
ELQANKATLVCLISDFYPGAVTVAWKADSSPVKAGVETTTPSKQSNNKYAASSYLSLTPEQWKS
HRSYSCQVTHEGSTVEKTVAPTECS

Binding domain B2:

AC_05059, light chain (SEQ ID NO: 388):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05059, heavy chain (SEQ ID NO: 389):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05060, light chain (SEQ ID NO: 390):
DIQMTQSPSSLSASVGDRVTITCRASQSIRDYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQGTFPFTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

AC_05060, heavy chain (SEQ ID NO: 391):
EVQLLESGGGLVQPGGSLRLSCAASGFTFGSYYMSWVRQAPGKGLEWVSGISGYGYYTGYADS
VKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARHGYGVIDYWGQGTLVTVSSASTKGPSVFP
LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS
SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT

PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY
KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN
GQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

AC_05061, light chain (SEQ ID NO: 392):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQGAYVPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05061, heavy chain (SEQ ID NO: 393):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYGYTHFDYWGQGTLVTVSSASTKGPSV
FPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

AC_05062, light chain (SEQ ID NO: 394):
DIQMTQSPSSLSASVGDRVTITCRASQAISGYLNWYQQKPGKAPKLLIYSASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQSYSTPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05062, heavy chain (SEQ ID NO: 395):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYRWHGSVFDYWGQGTLVTVSSASTKGP
SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05064, light chain (SEQ ID NO: 396):

DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQYPWYFPYTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

AC_05064, heavy chain (SEQ ID NO: 397):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSAISGSGGSTYYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYGYSVLDYWGQGTLVTVSSASTKGPSVF
PLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPS
SSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISR
TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE
YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWES
NGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPG
K

AC_05079, light chain (SEQ ID NO: 398):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05079, heavy chain (SEQ ID NO: 399):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPPLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05081, light chain (SEQ ID NO: 400):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05081, heavy chain (SEQ ID NO: 401):

EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFQPHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05088, light chain (SEQ ID NO: 402):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05088, heavy chain (SEQ ID NO: 403):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVLFPPHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05089, light chain (SEQ ID NO: 404):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05089, heavy chain (SEQ ID NO: 405):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPHHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE

WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05090, light chain (SEQ ID NO: 406):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05090, heavy chain (SEQ ID NO: 407):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPLHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05091, light chain (SEQ ID NO: 408):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05091, heavy chain (SEQ ID NO: 409):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPHFDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05093, light chain (SEQ ID NO: 410):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA

SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05093, heavy chain (SEQ ID NO: 411):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPHVDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05094, light chain (SEQ ID NO: 412):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05094, heavy chain (SEQ ID NO: 413):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFPPHWDYWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

AC_05096, light chain (SEQ ID NO: 414):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05096, heavy chain (SEQ ID NO: 415):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFRPHLDYWGQGTLVTVSSASTKG

PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

AC_05097, light chain (SEQ ID NO: 416):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQAGNPHTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTA
SVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYA
CEVTHQGLSSPVTKSFNRGEC

AC_05097, heavy chain (SEQ ID NO: 417):
EVQLLESGGGLVQPGGSLRLSCAASGFTFSSSYMGWVRQAPGKGLEWVSSIGSGSYSTSYAD
SVKGRFTISRDNSKNTLYLQMNSLRAEDTAVYYCARYPSVPFSPHLDYWGQGTLVTVSSASTKG
PSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVT
VPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLM
ISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLN
GKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE
WESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLS
LSPGK

Fab1, light chain (SEQ ID NO: 418):
DIQMTQSPSSLSASVGDRVTITCRASQSISSYLNWYQQKPGKAPKLLIYAASSLQSGVPSRFSG
SGSGTDFTLTISSLQPEDFATYYCQQSSHGPLLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGT
ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVY
ACEVTHQGLSSPVTKSFNRGEC

Fab1, heavy chain (SEQ ID NO: 419):
QVQLVQSGAEVKKPGSSVKVSCKASGGTFGYYAIHWVRQAPGQGLEWMGGIGSIFGTANYAQ
KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARAWSSDHMDYWGQGTLVTVSSASTKGPS
VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVP
SSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMIS
RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG
KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW
ESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLS
PGK

Fab3, light chain (SEQ ID NO: 420):
EIVLTQSPGTLSLSPGERATLSCRASQSVSSSYLAWYQQKPGQAPRLLIYGASSRATGIPDRFSG
SGSGTDFTLTISRLEPEDFAVYYCQQYWYPLTFGQGTKLEIKRTVAAPSVFIFPPSDEQLKSGTAS
VVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYAC
EVTHQGLSSPVTKSFNRGEC

Fab3, heavy chain (SEQ ID NO: 421):
QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSSSIHWVRQAPGQGLEWMGHIYPSFGTANYAQ
KFQGRVTITADESTSTAYMELSSLRSEDTAVYYCARHSGSRFFSPMDYWGQGTLVTVSSASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSV
VTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDT
LMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDW
LNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV
EWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL
SLSPGK

Example 1 – Isolation of CEACAM5 binders

Aim and background

The aim of the studies were to isolate anti human CEACAM5 binding domains.

Materials and methods

The studies included the use of two different phage display antibody libraries AlligatorGOLD® and AlligatorFAB. Isolation of binders was performed using phage display using cells displaying the antigens or with biotinylated antigens bound on magnetic beads. Screening of binders was performed using high-throughput (HT) ELISA, FACS or next generation sequencing (NGS).

<u>Phage selections:</u>

Phage display selections were performed, using either AlligatorGold® or the AlligatorFab libraries, according to selection strategies shown in Table 1. An overview of the different parameters used in the selections rounds during the phage selection process can be seen in Table 2 (Alligator GOLD, soluble antigen), Table 3 and Table 4 (Alligator GOLD, cell selections) or Table 5 (AlligatorFab libraries, soluble antigen). Overall, the starting libraries were thermally challenged at 45°C for 1h to ensure that the starting library only contained temperature stable antibody variants. Negative selection steps were added to remove potentially sticky binders as well as binders that

were reactive towards the presence of a Fc-part (using ubiquitin -His and CTLA-4-Fc). In addition, pre-selections against other CEACAM family members were performed, to ensure the specificity of the developed clones. The selection strategy was also designed to promote the isolation of clones with high affinity by stepwise decreasing the antigen concentration and increasing the number of wash steps. CEACAM5 from commercial sources was used as soluble antigen (R&D Systems, #4128-CM-050).

Table 1. Description of the different selections strategies and tracks performed

Type of phage selections	Antibody library	Selection Tracks	Pre-selection	Selection antigen
Biotinylated antigen on Dyna beads	AlligatorGOLD	1	CEACAM1, CEACAM6, CEACAM7, CEACAM8	CEACAM5
Cells overexpressing target		1	Yes, CEACAM1+ cells	CEACAM1/5$^+$ cells
		2	Yes, CEACAM1+ cells	Splice variant CEACAM5$^+$ cells
		3	Yes, CEACAM1+ cells	Full-length CEACAM5$^+$ cells
		3b	Yes, CEACAM1+ cells	Full-length CEACAM5$^+$ cells
Biotinylated antigen on Dyna beads	AlligatorFAB libraries	1	CEACAM1	CEACAM5

Table 2. AlligatorGOLD selections, soluble antigen - Selection parameters

Selection Round	Antigen conc	Thermal step	Selection time	Wash	Phage input
R#1	100 nM	Yes (45°C, 1 h)	1 h	5X 2 min	500 ul
R#2	10 nM	No	1 h	7X 2 min	500 ul
R#3	10 nM	Yes (50°C, 1h)	1 h	7X 2 min	500 ul
R#4	1 nM	No	20 min	7X 2 min + 1x 30 min	300 ul
R#5	0.1 nM	No	20 min	7X 2 min + 1x 30 min	300 ul
R#6	0.01 nM	No	20 min	7X 2 min + 1x 30 min rsh	300 ul

Table 3. Cell selections - Selection parameters Track 1-3

Selection Round	Antigen conc	Thermal step	Selection time	Wash	Phage input
R#1	1-5x10^6 cells	Yes (45°C, 1 h)	2 h	5X 2 min	500 ul

R#2	1-5x10^6 cells	No	1.5 h	8X 2 min PBST/ 2% BSA, 2X 2 min PBST	500 ul
R#3	1-5x10^6 cells	No	1.5 h	8X 2 min PBST/ 2% BSA, 2X 2 min PBST	500 ul
R#4	1-5x10^6 cells	No	1.5 h	8X 2 min PBST/ 2% BSA, 2X 2 min PBST	300 ul

Table 4. Cell selections - Selection parameters Track 3b

Selection Round	**Antigen conc**	**Thermal step**	**Selection time**	**Wash**	**Phage input**
R#1	1-5x10^6 cells	Yes (45°C, 1 h)	2 h	5X for 2 min	500 ul
R#2	1-5x10^6 cells	No	1 h	5X for 2 min	500 ul
R#3	1-5x10^6 cells	Yes (50°C, 1h)	1 h	5X for 2 min	500 ul
R#4	1-5x10^6 cells	No	1.5 h	8X 2 min PBST/ 2% BSA, 2X 2 min PBST	300 ul
R#5	1-5x10^6 cells	No	1.5 h	8X 2 min PBST/ 2% BSA, 2X 2 min PBST	300 ul
R#6	1-5x10^6 cells	No	1.5 h	8X 2 min PBST/ 2% BSA, 2X 2 min PBST	

Table 5. Alligator Fab selections - Selection parameters

Selection Round	**Antigen conc**	**Thermal step**	**Selection time**	**Wash**	**Elution**	**Phage input**
R#1	100 nM	Yes (45°C, 1 h)	1 h	5X 30s	Trypsin	500 ul or 950 µl
R#2	10 nM	No	1 h	5X 30s	trypsin	650 ul
R#3	1 nM	No	1 h	7X 30s	Trypsin	300 ul
R#4	0.1 nM or 1nM	No	1 h	7X 30s	Trypsin	300 ul
R#5	0.01 nM or 0.1nM	No	1 h	5X 30s or 7X 30s	Trypsin	300 ul

Screening of clones with ELISA

Screening was made on binders displayed on phages (test screenings) or using soluble scFv/Fab in bacterial supernatants. Briefly phage ELISAs were performed using CEACAM5 (#4128-CM-050, R&D Systems) or CTLA-4 Fc coated on microtiter plates to capture scFv/FAb displaying phages. Bound phages were detected with HRP conjugated monoclonal anti-M13 antibody (#27-9421-001, GE Healthcare). Positive phage clones were sequenced (Sanger sequencing at GATC). Unique phage clones were cloned to soluble scFv/Fab format. ELISA with soluble scFv/FAb were performed using the same antigens. Soluble scFv/Fab fragments were produced from bacterial TOP10 cultures. Unpurified supernatants were tested for binding towards antigen. Detection was made with HRP conjugated monoclonal anti-Flag antibody (#A8592, Sigma).

Screening of clones with FACS

Genes coding for human CEACAM5, human CEACAM1 or a chimera of CEACAM5 and CEACAM1 (CEACAM1/5) were cloned into pcDNA3.1, and stably transfected into CHO cells. FACS screening was performed using either CHO cells transiently transfected for CEACAM5 expression, or control wild type CHO cells, diluted in FACS buffer (DPBS/ 0.5% BSA). Cells were seeded (150 000 cells/ well) on to 96 well microtiter PP plates (#351190, Falcon). Supernatants of soluble Fab domains free of bacteria through centrifugation were diluted 1:1 in FACS buffer and added (50 µl) to seeded CHO cells. After incubation at 4 °C for 90 minutes cells were washed repeatedly with FACS buffer and resuspended in solution containing secondary antibody (α-hIgG F(ab')-PE, #19-116-097, Jackson ImmunoResearch) diluted at 1:1000 ratio. Following incubation for 1 hour at 4 °C cells were washed in FACS buffer and resuspended in Cellfix solution (#340181, BD) and signals were measured in a flow cytometer (BD).

Screening of clones with Next Generation Sequencing (NGS)

Purified DNA from Phage pools from rounds #4, #5 and #6 from the AlligatorGOLD® selections with soluble antigen was used to amplify scFV encoding DNA in PCR using primers (as listed in Table 6). The PCR was performed using Pfu Ultra II Hotstart PCR Master Mix (#600850, Agilent Technologies). 1 ng of DNA template was denatured at 98 °C for 30 seconds before DNA was amplified in 15 or 18 cycles of PCR reactions (98 °C for 30s, 58 °C for 30s and 72 °C for 30s) and an elongation phase of 2 minutes at 72 °C. Material was purified from agarose gel and further tagged with DNA barcode primers (listed in Table 6). 1 ng of DNA template was denatured at 98 °C for 30 seconds before DNA was amplified in 15 or 18 cycles of PCR reactions (98 °C for 10s, 68 °C for 30s and 72 °C for 31s) and an elongation phase of 2 minutes at 72 °C. DNA was purified (Purify DNA with GeneRead Size Selection kit, #180514 Qiagen) before sent for Illumina sequencing at the SciLife facility NGI, Stockholm. Data analyses included

quality control of obtained sequences and trimming of genes. Top candidates were aligned to sequences from clones isolated with traditional ELISA screening.

Table 6. PCR primers for generation of NGS libraries ('N' can correspond to any of A, T, C, or G)

Purpose of PCR reaction	**Type of primer**	**Sequence (5'- to 3')**
DNA amplification	forward primer	ACACTCTTTCCCTACACGACGCTCTTCCGATCTNNNNNCCTCTC CTGTGCAGCCAGCGG (SEQ ID NO: 373)
	reverse primer	AGACGTGTGCTCTTCCGATCTCTCCAGCTTGGTCCCCTGGCC (SEQ ID NO: 374)
Applying NGS barcodes	forward primer	AATGATACGGCGACCACCGAGATCTACACTAGATCGCACACTCT TTCCCTACACGACG (SEQ ID NO: 375)
	forward primer	AATGATACGGCGACCACCGAGATCTACACCTCTCTATACACTCT TTCCCTACACGACG (SEQ ID NO: 376)
	reverse primer	CAAGCAGAAGACGGCATACGAGATTCGCCTTAGTGACTGGAGT TCAGACGTGTGCTCTTCCGATCT (SEQ ID NO: 377)

Results and conclusions

The phage selections and screening resulted in the isolation of 38 binding domains against human CEACAM5. 29 clones originated from the AlligatorGOLD® library and 11 clones from the AlligatorFAB libraries.

Example 2 – Characterization of CEACAM5 binding scFv

Aim and background

The aim of the studies listed in this experiment was to further characterize the CEACAM5 binding single chain fragments (scFv) isolated in Example 1.

Materials and methods

The scFv were cloned in to IgG1 format and produced from mammalian cultures. Antibodies were analyzed in a set of different assays to test quality, and binding capacity towards CEACAM5 and related CEA family protein CEACAM1.

Expression and QC analyses

IgG1 antibodies listed in Table 7 and Table 8, and expression control monoclonal antibody 1188, were transiently expressed using Expi293 HEK (Life Technologies) cells at volumes ranging from 600µl to 30mL according to manufacturer's protocol. Purification of the antibodies from supernatants was made on protein A using the NGC system (BioRad) or Predictor MabSelectSure 50µl 96 well plates (GE Healthcare).

Aggregation was measured with SE-HPLC in a 1260 Infinity II system (Agilent Technologies) using a TSK gel Super SW mAB HTP 4µm, 4.6x150mm column (TOSOH Bioscience) and 100 mM Sodium Phosphate, pH 6.8, 300mM NaCl as mobile phase at ambient temperature and a flow rate of 0.35 ml/min.

CEACAM5 cell binding

CHO cells stably transfected, with pcDNA3.1 carrying either genes for human CEACAM5, human CEACAM1 or CEACAM5/CEACAM1 chimeras, and CHO wild type (wt) cells were incubated with 0.5 and 5 µg/ml IgG1 antibodies or for selected samples titrated antibody concentrations ranging from 67 nM to 0.4 pM. Binding of the antibodies was detected using fluorochrome-conjugated anti-human IgG and analysed using flow cytometry.

ELISA, CEA binding and cross-reactivity

96-well plates were coated with 0.5 µg/mL antigen, hCEACAM-5 (4128-CM-050, R&D Systems), hCEACAM-1 (2244-CM-050, R&D Systems), hCEACAM-6 (3934-CM-050, R&D Systems) or CEACAM-8 (9639-CM-050, R&D Systems) in PBS over night at 4°C. After washing with PBS/0.05% Tween 20 (PBST), the plates were blocked with PBST, 2% BSA for at least 30 minutes at room temperature before a second round of washing. Antibody samples, diluted to either 0.4 µg/ml, or 10 µg/ml for binders isolated with NGS, or for selected samples titrated from 67 nM to 0.4 pM in PBST, 0.5% BSA, were then added and allowed to bind for at least 1 hour at room temperature. After washing, plates were incubated with 50µl detection antibody (0.5µg/ml HRP conjugated goat anti human-kappa light chain, #STAR127P, AbD Serotec). Finally, a final round of washing was performed and bound complexes detected using SuperSignal Pico Luminescent as substrate and luminescence signals were measured using Fluostar Optima.

Octet binding studies

Kinetic measurements were performed in the Octet RED96 platform using several different setups. Monoclonal antibodies (listed in Table 9), serially diluted ½ starting at 100 nM, were captured to anti human IgG Fc Capture (AHC) Biosensor tips (Sartorius #18-5060). Human CEACAM5-His (R&D Systems #4128-CM-050) diluted in 1x kinetic buffer (Sartorius) at 100nM was then added. Binding kinetics was studied in 1x kinetic buffer where association was allowed for 300 sec followed by dissociation for 600 sec. Sensor tips were regenerated with 10mM Glycine pH 1.7. Data generated were referenced by subtracting blank or parallel buffer blank, the baseline was aligned to the y-axis, inter-step correction by alignment against dissociation was performed and

the data was smoothed by Savitzky-Golay filter in the data analysis software (v9.0.0.14). The processed data was fitted using a 1:1 Langmuir binding model with R^2 or X^2 as a measurement of fitting accuracy.

Results and conclusions

Results (summarized in Table 7) demonstrated the phage selection led to the isolation of several antibodies that displayed specific binding toward human CEACAM5. In particular antibodies AC_05059, AC_05060, AC_05061, AC_05062, AC_05064 showed a promising binding profile with selective binding towards human CEACAM5 as demonstrated both in ELISA (against CEACAM5, CEACAM1, CEACAM6, CEACAM7 and CEACAM8) as well as on FACS (with cells expressing CEACAM5, CEACAM1 or a CEACAM1/5 chimera).

In addition, the results also show that clones selected using next generation screening also generated clones with specific binding toward human CEACAM5 as demonstrated in an ELISA study toward CEACAM5 and CEACAM1 (Table 8 and Figure 1). In particular antibodies AC_05079, AC_05081, AC_05088, AC_05089, AC_05090, AC_05091, AC_05093, AC_05094, AC_05096 and AC_05097 show selective binding for human CEACAM5.

Affinity measurements with Octet demonstrated the analysed antibodies displayed a range of different affinities toward CEACAM5 (Table 9).

Table 7 ._Binding studies with CEA binders in IgG1 format

AC-name	HPLC (% mono)	Binding to CEACAM5 on cells	Binding to CEACAM1 on cells	Binding to CEACAM1/5 chimera on cells	Cross-reactivity to other CEA family members
AC_05066	97'1	yes	yes	yes	-
AC_05068	97'7	no	no	no	-
AC_05069	97'2	yes	yes	yes	-
AC_05070	97'9	yes	yes	yes	-
AC_05071	98'8	yes	yes	yes	-
AC_05073	99'3	yes	yes	yes	-
AC_05074	95'8	yes	yes	yes	-
AC_05075	96'8	yes	yes	yes	-
AC_05076	94	yes	yes	yes	-
AC_05077	96'6	yes	yes	yes	-
AC_05078	98'6	weak	yes	yes	-
AC_05059	90'6	yes	weak	weak	OK

AC_05060	98'8	yes	no	no	OK
AC_05061	97'3	yes	no	no	OK
AC_05062	98'1	yes	no	no	OK
AC_05064	84'3	yes	no	no	OK
AC_05065	94'2	no	no	no	-

Table 8. Binding towards CEACAM5 and CEACAM1for antibodies isolated using NGS as shown in ELISA.

	CEACAM5	CEACAM1
AC_05079	Yes	No
AC_05080	Yes	Weak
AC_05081	Yes	No
AC_05082	No	No
AC_05083	Yes	Yes
AC_05085	No	No
AC_05086	Yes	Yes
AC_05087	Yes	Yes
AC_05088	Yes	No
AC_05089	Yes	No
AC_05090	Yes	No
AC_05091	Yes	No
AC_05092	No	No
AC_05093	Yes	No
AC_05094	Yes	No
AC_05095	Yes	Weak
AC_05096	Yes	No
AC_05097	Yes	No
AC_05098	No	No
AC_05099	No	No
AC_05100	No	No

Table 9. Affinity measurement against CEACAM5 for antibodies in IgG1 format

Sample	KD (M)	kon(1/Ms)	kdis(1/s)
AC_05059	<1.0E-12	3E+05	<1.0E-07
AC_05060	6E-09	4E+05	2E-03
AC_05061	2E-08	1E+05	2E-03
AC_05062	9E-10	*2E+06*	*2E-03*
AC_05064	4E-10	1E+05	6E-05
AC_05080	8E-12	2E+05	2E-06
AC_05081	1E-07	6E+04	8E-03
AC_05088	2E-08	1E+06	3E-02
AC_05089	4E-08	3E+05	1E-02
AC_05090	2E-08	3E+05	4E-03

AC_05091	2E-09	2E+05	3E-04
AC_05093	4E-10	3E+05	1E-04
AC_05094	5E-08	2E+04	8E-04
AC_05097	7E-11	2E+05	2E-05

Example 3 – Generation and manufacturability of bispecific antibodies targeting CD40 and CEACAM5

Background and aim

Bispecific antibodies (bsAbs) were generated by combining above evaluated CEACEAM5 targeting antibodies with any of the CD40 agonistic antibodies 1132, G12 or 'G12_mut' in the RUBY™ format. In brief, bispecific antibodies in the RUBY™ format are generated by appending the antigen-binding fragments (Fab) of an antibody to the c-terminal part of the heavy chain of an IgG via a short peptide linker. A selected set of bsAbs were further transferred into a variant of the RUBY™ format with optimized properties. Generated RUBY™ bsAbs are listed in Table 10. Manufacturability of the listed bsAbs targeting CD40 and CEACAM5 was evaluated in terms of production yields and purity after protein A purification.

Materials and methods

RUBY™ bsAbs listed in Table 10, and expression control monoclonal antibody 1188, were transiently expressed using Expi293 HEK (Life Technologies) cells at volumes ranging from 600µl to 30mL according to manufacturer's protocol. Cells were transfected with three different vectors each encoding one of the three polypeptide chains of RUBY™ bsAbs (i.e., the immunoglobulin heavy chain linked to the linker and Fab light chain (Chain H1), the immunoglobulin light chain (Chain L1) and the Fab heavy chain (Chain H2)). Purification of the antibodies from supernatants was made on protein A using the NGC system (BioRad) or Predictor MabSelectSure 50µl 96 well plates (GE Healthcare). Different transfection ratios of the three vectors were tested. Aggregation was measured with SE-HPLC in a 1260 Infinity II system (Agilent Technologies) using a TSK gel Super SW mAB HTP 4µm, 4.6x150mm column (TOSOH Bioscience) and 100 mM Sodium Phosphate, pH 6.8, 300mM NaCl as mobile phase at ambient temperature and a flow rate of 0.35 ml/min.

Results and conclusions

Bispecific antibodies could be generated in the RUBY™ format by combining CD40 and CEACAM5 targeting antibodies. Table 11, shows the production yields from high-throughput transient cultures and the monomer fraction as measured by SE-HPLC after protein A high-throughput purification. Generally good productivity and quality was

observed. In conclusion, it is possible to generate and produce RUBY™ bsAbs targeting CD40 and CEA of high purity.

Table 10. List of generated RUBY™ bsAbs.

RUBY™ ID	mAb origin		Target combination	Format
	IgG position	Fab position		
Multi1	G12	Fab1	CD40xCEACAM5	RUBY™, *
Multi2	G12	Fab2	CD40xCEACAM5	RUBY™, *
Multi3	G12	Fab3	CD40xCEACAM5	RUBY™, *
Multi4	G12	Fab4	CD40xCEACAM5	RUBY™, *
Multi5	G12	Fab5	CD40xCEACAM5	RUBY™, *
Multi6	G12	Fab6	CD40xCEACAM5	RUBY™, *
Multi7	G12	Fab7	CD40xCEACAM5	RUBY™, *
Multi8	G12	Fab8	CD40xCEACAM5	RUBY™, *
Multi9	G12	Fab9	CD40xCEACAM5	RUBY™, *
Multi10	G12	Fab10	CD40xCEACAM5	RUBY™, *
Multi11	G12	Fab11	CD40xCEACAM5	RUBY™, *
Multi12	Fab1	G12	CEACAM5xCD40	RUBY™, *
Multi13	Fab2	G12	CEACAM5xCD40	RUBY™, *
Multi14	Fab3	G12	CEACAM5xCD40	RUBY™, *
Multi17	Fab6	G12	CEACAM5xCD40	RUBY™, *
Multi18	Fab7	G12	CEACAM5xCD40	RUBY™, *
Multi19	Fab8	G12	CEACAM5xCD40	RUBY™, *
Multi20	Fab9	G12	CEACAM5xCD40	RUBY™, *
Multi23	1132	Fab1	CD40xCEACAM5	RUBY™, *
Multi24	1132	Fab2	CD40xCEACAM5	RUBY™, *
Multi25	1132	Fab3	CD40xCEACAM5	RUBY™, *
Multi26	1132	Fab4	CD40xCEACAM5	RUBY™, *
Multi27	1132	Fab5	CD40xCEACAM5	RUBY™, *
Multi28	1132	Fab6	CD40xCEACAM5	RUBY™, *
Multi29	1132	Fab7	CD40xCEACAM5	RUBY™, *
Multi30	1132	Fab8	CD40xCEACAM5	RUBY™, *
Multi31	1132	Fab9	CD40xCEACAM5	RUBY™, *
Multi32	1132	Fab10	CD40xCEACAM5	RUBY™, *
Multi33	1132	Fab11	CD40xCEACAM5	RUBY™, *
Multi34	G12_mut	Fab1	CD40xCEACAM5	Optimized RUBY™, §
Multi35	G12_mut	Fab2	CD40xCEACAM5	Optimized RUBY™, §
Multi37	G12_mut	Fab6	CD40xCEACAM5	Optimized RUBY™, §
Multi38	G12_mut	Fab8	CD40xCEACAM5	Optimized RUBY™, §
Multi39	G12_mut	Fab10	CD40xCEACAM5	Optimized RUBY™, §
Multi40	Fab1	G12_mut	CEACAM5xCD40	Optimized RUBY™
Multi41	Fab2	G12_mut	CEACAM5xCD40	Optimized RUBY™
Multi42	Fab3	G12_mut	CEACAM5xCD40	Optimized RUBY™

Multi44	Fab7	G12_mut	CEACAM5xCD40	Optimized RUBY™
Multi46	1132	Fab1	CD40xCEACAM5	Optimized RUBY™, §
Multi47	1132	Fab2	CD40xCEACAM5	Optimized RUBY™, §
Multi48	1132	Fab8	CD40xCEACAM5	Optimized RUBY™, §
Multi49	1132	Fab10	CD40xCEACAM5	Optimized RUBY™, §
AC_05333	G12	5090	CD40xCEACAM5	RUBY™, *
AC_05334	G12	5093	CD40xCEACAM5	RUBY™, *
AC_05336	5088	G12	CEACAM5xCD40	RUBY™, *
AC_05337	5090	G12	CEACAM5xCD40	RUBY™, *
AC_05338	5093	G12	CEACAM5xCD40	RUBY™, *
AC_05339	5097	G12	CEACAM5xCD40	RUBY™, *
AC_05341	1132	5090	CD40xCEACAM5	RUBY™, *
ffAC_05337	5090	G12_mut	CEACAM5xCD40	Optimized RUBY™, §
ffAC_05339	5097	G12_mut	CEACAM5xCD40	Optimized RUBY™, §
AC_05355	mAb2	G12	CEACAM5xCD40	RUBY™, *

* Carries the following mutations: in Chain H1 Q44R (according to IMGT numbering system) in the VH of the IgG; H168A, F170G (according to the Eu numbering system) in the CH1; Q44R (according to IMGT numbering system) in the VL; and S114A, N137K (according to the Eu numbering system) in CKappa of the appended Fab position; in Chain L1 Q44E (according to the IMGT numbering system) in the VL; and L135Y, S176W (according to the Eu numbering system) in Ckappa; in Chain H2 Q44E (according to the IMGT numbering system) in the VH; and T187E (according of Eu numbering system) in the CH1.

§ Additional mutations included: T109P (according to the Eu numbering system) in the CKappa of the appended Fab.

Table 11. Expression volume, production yield and purity of CD40 and CEACAM5 targeted bsAbs

RUBY™ ID	**Expression vol.**	**Yield (mg/L)**	**Monomeric fraction (%)**
Multi1	600µl	67	96
Multi2	600µl	66	97
Multi3	600µl	58	97
Multi4	600µl	62	100
Multi5	600µl	55	98
Multi6	600µl	84	97
Multi8	600µl	124	88
Multi9	600µl	85	92
Multi10	600µl	82	95
Multi11	600µl	67	96

Multi13	600µl	24	85
Multi14	600µl	40	94
Multi17	600µl	14	100
Multi18	600µl	11	93
Multi19	600µl	56	93
Multi20	600µl	64	85
Multi23	600µl	29	93
Multi24	600µl	29	92
Multi25	600µl	25	94
Multi26	600µl	29	99
Multi27	600µl	27	90
Multi28	600µl	22	93
Multi29	600µl	26	80
Multi30	600µl	107	83
Multi31	600µl	85	86
Multi32	600µl	39	91
Multi33	600µl	98	88
Multi34	30ml	18	96
Multi35	30ml	79	96
Multi37	30ml	20	97
Muli38	30ml	126	87
Multi39	30ml	101	92
Multi40	30ml	95	85
Multi41	30ml	67	84
Multi42	30ml	16	100
Multi44	30ml	20	93
Multi46	30ml	79	94
Multi47	30ml	91	92
Multi48	30ml	152	83
Multi49	30ml	110	82
AC_05333	30ml	112	90
AC_05334	30ml	107	81
AC_05336	30ml	15	99
AC_05337	30ml	29	97
AC_05338	30ml	30	97
AC_05339	30ml	34	97
AC_05341	30ml	73	81
ffAC_05337	30ml	33	98
ffAC_05339	30ml	72	96
AC_05355	30ml	225	96
1188	600µl	104-172	97-99
1188	30ml	70-180	99

Example 4 – Dual antigen binding and binding to CEACAM variants of CD40 and CEACAM5 targeting bsAbs by ELISA

Aim

The aim of the study was to evaluate the ability of selected CD40 and CEACAM5 targeting RUBY™ bsAbs to bind both their targets simultaneously as well as their potential cross-reactivity with additional members of the CEA protein family was evaluated by ELISA.

Materials and Methods

96-well plates were coated with 0.5 µg/mL antigen, hCEACAM-1 (2244-CM-050, R&D Systems), hCEACAM-5 (4128-CM-050, R&D Systems), hCEACAM-6 (3934-CM-050, R&D Systems) or CEACAM-8 (9639-CM-050, R&D Systems) in PBS over night at 4°C. After washing in PBS/0.05% Tween 20 (PBST), the plates were blocked with PBST, 2% BSA for at least 30 minutes at room temperature before a second round of washing. RUBY bsAbs, diluted in PBST, 0.5% BSA, were then added and allowed to bind for at least 1 hour at room temperature. After washing, plates were incubated with either 50µl detection antibody (0.5µg/ml HRP conjugated goat anti human-kappa light chain, #STAR127P, AbD Serotec) for analysis of binding to CEACAM protein family proteins or 0.5µg/ml biotinylated hCD40-muIg (504-030, Ancell) followed by HRP conjugated streptavidin (21126, Pierce) for confirmation of dual antigen binding. Finally, a final round of washing was performed and bound complexes detected using SuperSignal Pico Luminescent as substrate and luminescence signals were measured using Fluostar Optima.

Results and Conclusions

All evaluated RUBY™ bsAbs was indeed able to bind to both CD40 and human CEACAM5 simultaneously (Figure *2*), although with varying potency. In general, bsAbs carrying 1132 as CD40 binding antibody (Multi46-Multi49) displayed lower potency in the dual target ELISA, as compared to bsAbs carrying G12_mut. Also, Multi38 displayed reduced dual target binding compared to other G12_mut based bsAbs, likely due to lower CEACAM5 binding of Fab6 than other evaluated CEACAM5 binding antibodies.

As can be seen in Figure *3*, a majority of the evaluated CD40 and CEACAM5 targeting RUBY™ bsAbs did not cross react with any of the other CEA family members evaluated. However, a limited number of the assayed bsAb did show significant cross-reactivity with CEACAM1 (Multi38, Multi39, Multi45 and Multi 49) or CEACAM6 (Multi40).

All in all, it can be concluded that all evaluated RUBY™ bsAbs have the ability to bind CD40 and CEACAM5 simultaneously and a majority of the set was specific for

CEACAM5, with no or little detectable binding to other evaluated members of the CEA protein family.

Example 5 – Kinetics of interaction between bispecific antibodies and CEACAM5 and CD40

Aim and background

The aim of these studies was to measure the binding affinities of selected CD40 x CEACAM5 targeting RUBY™ bsAb using several different assay set ups to obtain a comprehensive understanding of the bsAbs binding kinetics. The assays included both set ups using immobilized CEACAM5 or CD40 or monomeric CEACAM5 or CD40 in solution.

Materials and methods

Kinetic measurements were performed in the Octet RED96 platform using several different setups. Bispecific antibodies (listed in Table 12 – Table 15) or biotinylated human CEACAM5-His (Acro Biosystems #CE5-H82E0) or biotinylated human CD40-mouse Fc (Ancell #504-030) were captured to anti human IgG Fc Capture (AHC) Biosensor tips (Sartorius #18-5060) or Streptavidin Biosensor tips (Sartorius #18-5019). Monomeric human CD40-His-Avi tag (Acro Biosystems #CD0-H5228), monomeric Human CEACAM5-His (R&D Systems #4128-CM-050) or monomeric Cynomolgus CEACAM5-His (Sino Biological #90891-C08H) were ½ serially diluted in 1x kinetic buffer (Sartorius) starting at 500nM or 100nM. Bispecific antibodies were ½ serially diluted starting at 50nM, 25nM or 10nM or 1/1.5 serially diluted starting at 15nM or 5nM. Binding kinetics was studied in 1x kinetic buffer where association was allowed for 100 sec, 300 sec or 600 sec followed by dissociation for 100 sec, 300 sec or 3600 sec. Sensor tips were regenerated with 10mM Glycine pH 1.7. Data generated were referenced by subtracting blank or parallel buffer blank, the baseline was aligned to the y-axis, inter-step correction by alignment against dissociation was performed and the data was smoothed by Savitzky-Golay filter in the data analysis software (v9.0.0.14). The processed data was fitted using a 1:1 Langmuir binding model with R^2 or X^2 as a measurement of fitting accuracy.

Results and conclusions

The two anti CD40 binding domains; 1132 and G12 interact with captured CD40 with K_D values in the nM range, but with different kinetic profiles (Table 12). The same anti CD40 binding domains in IgG position or in Fab position in the bispecific antibody interact with similar affinity and kinetics to CD40 (Table 12). A summary of kinetic

profiles for the CEACAM5 interactions measured in the Octet is listed in Table 13 and Table 14. Example of sensograms of soluble monomeric human CEACAM5 interacting with captured bsAb is shown in Figure *4*. Example of bsAb in solution interacting with capture CEACAM5 is shown in Figure *5*. The kinetic profiles and affinities for the bsAb interacting with human CEACAM5 varies with K_D from higher nM range to sub nM range. The cynomolgus CEACAM5 reactive bsAb AC_05355 were interacting with both human CEACAM5 and cynomolgus CEACAM5 with K_D in the nM range (Table 15).

Table 12 Summary of kinetic profiles measured in Octet. Bispecific antibodies in solution (25-0.8 nM ,10-2 or 5-0.7nM.) interacting with captured CD40mFc-biotin. Association was measured for 300 sec or 600 sec and dissociation was measured for 300 sec or 3600 sec

Bispecific antibody	**BsAb**		**k_a(1/Ms)**	**k_d(1/s)**	**K_D (M)**	**R^2**
	IgG	**Fab**				
AC_05293	1132	control 3174 (comprising SEQ ID NOs: 422 and 424)	8.3E+03	6.2E-04	7.6E-08	0.96
AC_05300	control 3174	1132	5.4E+05	4.6E-03	8.5E-09	0.97
AC_05330	G12	control 1210 (comprising SEQ ID NOs: 426 and 428)	3.5E+04	1.6E-05	4.5E-10	1.00
AC_05331	control 1210	G12	3.6E+04	8.1E-06	2.3E-10	1.00

Table 13 Summary of kinetic profiles for soluble monomeric CEACAM5 (100-1.6 nM) interacting with captured BsAb in Octet. Association was measured for 100 sec and dissociation was measured for 100 sec.

Bispecific antibody	BsAb		k_a(1/Ms)	k_d(1/s)	K_D (M)	R^2
	IgG	Fab				
Multi34	G12_mut	Fab1	2.2E+05	<1.0E-07	<1.0E-12	0.91
Multi35	G12_mut	Fab2	1.6E+05	<1.0E-07	<1.0E-12	0.93
Multi42	Fab3	G12_mut	4.7E+05	9.1E-04	2.0E-09	1.00
Multi44	Fab7	G12_mut	1.8E+05	2.3E-03	1.3E-08	0.98
Multi46	1132	Fab1	1.3E+05	<1.0E-07	<1.0E-12	0.94
Multi47	1132	Fab2	6.3E+02	1.1E-03	1.8E-06	0.82
Multi48	1132	Fab8	2.4E+04	<1.0E-07	<1.0E-12	0.83
Multi49	1132	Fab10	2.8E+04	<1.0E-07	<1.0E-12	0.76
AC_05339	5097	G12	2.4E+05	4.2E-04	1.8E-09	0.99

Table 14. Summary of kinetic profiles of BsAb (100-1.6nM or 50-0.8nM) interacting with captured CEACAM5-biotin in Octet. Association was measured for 100 sec and dissociation measured for 100 sec.

Bispecific antibody	BsAb		k_a(1/Ms)	k_d(1/s)	K_D (M)	R^2
	IgG	Fab				
Multi34	G12_mut	Fab1	4.0E+03	1.1E-04	2.8E-08	0.98
Multi35	G12_mut	Fab2	2.8E+03	3.7E-04	1.3E-07	0.94
Multi37	G12_mut	Fab6	1.9E+03	2.6E-03	1.4E-06	0.93
Multi41	Fab2	G12_mut	4.7E+04	<1.0E-07	<1.0E-12	0.97
Multi42	Fab3	G12_mut	1.8E+03	1.5E-03	8.4E-07	0.97
Multi44	Fab7	G12_mut	1.5E+05	<1.0E-07	<1.0E-12	1.00
Multi46	1132	Fab1	6.7E+04	1.3E-04	1.9E-09	1.00

Table 15. *Summary kinetic profile for soluble human CEACAM5 (100-1.6nM) or cynomolgus CEACAM5 (500-8 nM) interacting with captured BsAb in Octet. Association was measured for 100 sec and dissociation for 300 sec.*

Bispecific Antibody	Antigen	k_a(1/Ms)	k_d (1/s)	K_D (M)	R^2
AC_05339	human CEACAM5	5.2E+05	<1.0E-07	<1.0E-12	0.99
AC_05355	human CEACAM5	5.1E+05	5.3E-04	1.0E-09	1.00
AC_05355	cyno CEACAM5	1.9E+05	3.8E-03	2.0E-08	1.00

Example 6 – Binding of CD40-CEACAM5 bispecific antibodies to CEACAM5 expressing cells

Aim and background

The aim of this study was to assess the binding of the CD40-CEA RUBY™ bispecific antibodies to CEACAM5 expressed on cells and evaluate potential cross-reactivity to CEACAM1. In this study both CEACAM5 transfected cells and human tumor cells with endogenous CEACAM5 expression were used.

Materials and methods

The human CEACAM5 and CEACAM1 genes were cloned into pcDNA3.1, and the vector was subsequently stably transfected into CHO cells. The tumor cell line MKN45, expressing high levels of CEACAM5, LS174T expressing intermediate levels of CEACAM5, and HT29 and LOVO expressing low levels of CEACAM5 (*Table* 16), CHO-CEACAM5, CHO-CEACAM1 and to CHO wt cells were incubated with titrated concentrations of CD40-CEA bispecific antibodies. Binding of the antibodies was

detected using fluorochrome-conjugated anti-human IgG and analyzed using flow cytometry.

Results and conclusions

The data demonstrate that all tested CD40-CEACAM5 RUBYs bind to CEACAM5 expressed on CHO-CEACAM5 (Figure 6A - Figure 6E), and MKN45 (high expressing) (Figure 8A – Figure 8C) and LS174T (intermediate expressing) human tumor cells (Figure 8D – Figure 8F). Low or no binding was observed to the CEACAM5 low expressing tumor cells, the LOVO cells (Figure 8G- Figure 8I). In addition, a low cross-reactivity to CEACAM1 or stickiness to CHO wt cells was observed with some of the CD40-CEA bispecific antibodies at very high concentrations (Figure *7*). In conclusion, all the CD40-CEA RUBY™ bispecific antibodies bind to CEACAM5 and with low or no binding to CEACAM1.

Table 16. Summary of CEA expression levels on CEACAM5 transfected CHO cells and CEA expressing human tumor cells.

Tumor cell line and CEA transfected CHO cells	**Receptors/cell**
HT29	11 300
LOVO	5 500
LS174T	51 500
MKN45	353 000
CHO-CEACAM5	125 000

Example 7– Evaluation of the CD40 agonistic function using the CD40 reporter assay.

Aim and background

The aim of this study was to assess the CD40 agonistic function of the CD40-CEACAM5 RUBY™ bispecific antibodies using the CD40 reporter assay in the presence of CEACAM5 expressing cells. CD40 crosslinking will be mediated by simultaneous binding of CD40, expressed on CD40 reporter cells, and CEACAM5 expressed on CHO cells or CEACAM5 expressing human tumor cells. In addition, since high levels of soluble CEACAM5 can be detected systemically in cancer patients, the agonistic function was also assessed in the presence of physiological relevant concentrations of soluble CEACAM5.

Materials and methods

Agonistic function of the CD40-CEACAM5 RUBYs was evaluated using a CD40 reporter assay (Promega, CD40 Bioassay Kit CS JA2155). The assay was performed according to the manufacturer's protocol. In brief, CD40 reporter cells and titrating

concentrations of CD40-CEACAM5 RUBYs were diluted in RPMI containing 10% FCS and added to the assay plates before the addition of CEACAM5 transfected CHO, CHO wt or CEA expressing human tumor cells. In addition, the assay was also performed in the presence of 0.5 or 5 ug/ml soluble CEA. The assay plates were incubated for 6 h at 37°C until addition of Bio-GloTM Luciferase Assay Detection solution and analyzed in the BMG ELISA plate reader.

Results and conclusions

The results show that all tested CD40-CEA bispecific antibodies induce CD40 activation in the presence of CEA (*Figure 9*), and the efficacy correlates to the CEA expression level as seen when the reporter cells were co-cultured with human tumor cells expressing different CEA levels (*Figure 10*). The majority of the evaluated CD40-CEA bispecific antibodies were unaffected by the presence soluble CEA in the cultures, except Multi35. The potency of Multi35 was decreased in the presence of soluble CEA (*Figure 11*).

Example 8 – Assessment of agonistic function of CD40-CEACAM5 RUBYs in the B cell assay

Aim and background

The aim of this study was to assess the effect of the CD40-CEACAM5 bispecific antibodies on B cell activation *in vitro* in the presence or absence of CEACAM5. CD40 crosslinking will be mediated by simultaneous binding of CD40, expressed on B cells, and CEACAM5 transfected CHO cells.

Materials and methods

The agonistic effect of CD40-CEACAM5 bispecific antibodies was assessed in a B cell assay, based on primary human B cells. Briefly, B cells were isolated from human peripheral blood mononuclear cells by MACS according to the manufacturer's protocol (Miltenyi Biotec #130-091-151). Human CEACAM5 transfected CHO cells, cynomolgus CEACAM5 transfected CHO cells or CHO wt cells were UV irradiated and seeded in tissue culture treated 96 well flat bottom plates (Eppendorf). B cells were cocultured with the CHO cells in the presence of IL-4 (10 ng/ml, Gibco #PHC0045) and titrated concentrations of CD40-CEACAM5 bispecific. After 2 days, B cells were harvested and expression level of the activation marker CD86 was analyzed by FACS.

Results and conclusions

The data demonstrate that tested CD40-CEA RUBYs induce upregulation of CD86 on B cells in the presence of CEA (*Figure 12*, *Figure 13*).

Example 9 – CD40 Sink assay

Aim and background

To mimic the CD40 sink effect from CD40 expressing cells in circulation, the functionality of the CD40xCEA bispecific antibodies were evaluated in presence of competing CD40 expressing cells.

Materials and methods

CHO-cells transfected with human CEACAM5 were used for crosslinking. The CEACAM5 gene was cloned into pcDNA3.1. The vector was subsequently stably transfected into CHO cells. The expression of CEACAM5 was confirmed by staining with commercial antibody targeting CD66e (Invitrogen #PA5-16665). Wild type CHO (CHO-wt) cells were used as control cells for absence of crosslinking.

HEK Blue™ CD40L cells (Invivogen hkb#40) were stably transfected with CD40 and an NF-κB-inducible secreted embryonic alkaline phosphatase (SEAP) construct. Binding to CD40 led to activation of NFκB and production of SEAP, which was monitored using QUANTI-Blue™ substrate (Invivogen #rep-qbs).

A HTS Transwell 96 well permeable support system containing a Transwell receiver plate (Corning #3382) and a Transwell insert with 0.4µm pore sized membrane (Corning #3391) was used.

HEK Blue CD40L cells (30 000 cells/well) and CHO-CEACAM5 cells or CHO-wt (5000 or 10 000 cells/well) were transferred to the receiver plate. The insert was placed onto the receiver plate. Buffer with increasing number of CD40 expressing sink cells, in this case Raji cells (0-100 000 cells/well), were transferred to the insert. Finally bispecific antibodies (bsAb), listed in Table 17, at one concentration or serially diluted 1/3 from 100nM or 3nM were added to the insert. Following 20-24h incubation the SEAP content in the culture medium in the receiver plate was measured using the QUANTI Blue™ substrate.

Results and conclusions

As shown in *Figure 14* a crosslinking dependent activation of HEK Blue CD40L cells was received for AC_05339. The activation was inhibited by addition of increasing number of Raji sink cells as seen in *Figure 14*, *Figure 15*, *Figure 16* and *Figure 17* The inhibition of activation became greater with increasing amount of CD40 sink cells present. A summary of EC50 is shown in Table 17. A 2-fold to 13-fold increase in EC50 was

measured when including 90 000 Raji cells. Similar effect was received on antiCD40 G12 in IgG and Fab position of RUBY™ bsAb. A possibly lower effect on antiCD40 1132 was observed. In conclusion, all BsAb's tested were functional in presence of CD40 expressing sink cells, but with a decreased potency.

Table 17. Summary of EC50 (nM) measured for BsAb (3-0.004nM or 100-0.1nM) as single samples crosslinked to CHO-CEACAM5 cells activating HEK Blue CD40L™ reporter cells w/o presence of Raji sink cells, monitored as release of SEAP into culture media, measured with QuantiBlue™.

BsAb	RUBY™ position		EC50 (nM)			
			Number of Raji sink cells/well			
	IgG	Fab	0	10 000	30 000	90 000
ffAC_05337	5090	G12	0.04	0.08	0.11	0.41
AC_05339	5097	G12	0.03	0.05	0.08	0.23
Multi34	G12	Fab1	0.03	0.06	0.09	0.33
Multi35	G12	Fab2	0.06	0.09	0.14	0.34
Multi41	Fab2	G12	0.03	0.06	0.13	0.36
Multi42	Fab3	G12	0.04	0.06	0.11	0.36
Multi44	Fab7	G12	0.02	0.05	0.08	0.16
Multi46	1132	Fab1	0.5	0.81	1.2	2.5
Multi47	1132	Fab2	4.4	5.7	7.2	10.3

Example 10 – Colocalization of CEACAM5$^+$ tumor debris and Raji cells induced by CD40-CEA RUBY

Aim and background

The aim of this study was to assess the colocalization of CEACAM5 expressing tumor cell debris and CD40 expressing Raji cells induced by CD40-CEA RUBY.

Materials and methods

A tumor cell line expressing CEACAM5 was stained with the fluorescent membrane dye PKH26 (Sigma-Aldrich) followed by heat shock at 45°C for 10 min to induce cell death. Heat-shocked tumor cells were incubated at 37°C overnight, spun down and supernatant containing tumor cell debris was collected.

Raji cells were labelled with the nuclear stain Hoechst 33342 (0.045 ug/ml, Thermo Fisher) and seeded in 96-well flat-bottom plates (Costar). Tumor cell debris and CD40-CEACAM5 (AC_05339) RUBY or CD40 (1132.m2) control mAb were added, and cells were imaged using Cytation5 (BioTek) every two hours. Gen5 software was used to analyze the number of colocalized tumor debris and Raji cells.

Results and conclusions

The results show increased colocalization of CEA expressing tumor cell debris and CD40 expressing Raji cells induced by the CD40-CEA RUBY compared to the CD40 monoclonal antibody (*Figure 18*).

Example 11: Anti-tumor effect and immunological memory induction of CD40-CEACAM5 bispecific antibodies

Background and aim

AC_05337 and AC_05339 are CD40-CEA bispecific antibodies in RUBY™ format which have been LALA-mutated to silence Fcg receptor binding.

The aim of this study was to evaluate the anti-tumor effect of AC_05337 and AC_05339 in human CD40 transgenic (hCD40tg) mice inoculated with murine MC38 tumors transfected with human CEA (MC38-CEACAM5), and to assess immunological memory formation in mice cured from the tumors by treatment with CD40-CEA bsAbs.

Materials and methods

Female hCD40tg mice of 10 weeks of age were inoculated with $1x10^6$ MC38-CEACAM5 cells subcutaneously (s.c.) in the right flank. On days 7, 10, and 13 after inoculation, the mice were administered intraperitoneally (i.p.) with 100 µg of wildtype CD40 monospecific antibody, G12, or 167 µg of the CD40-CEA bsAbs AC_05337 and AC_05339. A group of vehicle-treated mice was also included. The tumors were frequently measured with a caliper in width (w), length (l) and height (h) and the tumor volume was calculated using the formula: (w/2 x l/2 x h/2 x π x (4/3)). Naïve hCD40tg control mice at 10 weeks of age and mice cured from the MC38-CEACAM5 tumors by treatment with AC_05339 (complete responders) were inoculated s.c. in the right flank with $1x10^6$ MC38-wt cells. The tumors were frequently measured with a caliper in width (w), length (l) and height (h) and the tumor volume was calculated using the formula: (w/2 x l/2 x h/2 x π x (4/3)).

Results and conclusions

The data demonstrate that treatment with the CD40-CEACAM5 bsAbs AC_05337 and AC_05339, but not the CD40 mAb G12, significantly reduces the MC38-CEA tumor volume compared to vehicle-treated mice (*Figure 19*). MC38-wt cells display tumor formation and growth in naïve mice, but not in complete responder mice. This suggests that the rechallenged mice have developed immunological memory against the MC38

tumor following treatment with the CD40-CEACAM5 bsAb AC_05339, and that this immunological memory is not restricted to CEACAM5 (*Figure 19*).

Example 12 – A 2 week toxicity study of AC_05355, a CD40xCEACAM5 targeting RUBY™ bsAb, in cynomolgus monkeys

Background and aim

To facilitate toxicity studies in cynomolgus monkeys a RUBY™ bsAb (AC_05355), cross-reactive between human and cynomolgus variants of CD40 and CEACAM5, was generated. AC_05355 carries mutations L234A, L235A in its Fc, rendering the bsAbs silenced in terms of Fcγ receptor binding and thus dependent on CEACAM5 engagement to stimulate DC40 mediated activation. After confirmation of *in vitro* and *in vivo* functionality, the potential toxicity of this bsAb targeting CD40 and CEACAM5, when given via once weekly intravenous infusion for 2 weeks to cynomolgus monkeys, was evaluated.

Material & Methods

In vitro functionality

The agonistic effect of AC_05355 was assessed in a B cell assay, based on primary B cells isolated from cynomolgus monkeys. Briefly, B cells were isolated from cynomolgus peripheral blood mononuclear cells by MACS according to the manufacturer's protocol (Miltenyi Biotec, #130-091-105). Cynomolgus CEACAM5 transfected CHO cells were UV irradiated and seeded in tissue culture treated 96 well flat bottom plates (Eppendorf). B cells were cocultured with CHO cells in the presence of IL-4 (10 ng/ml, Gibco #PHC0045) and titrated concentrations of AC_05355. After 2 days, B cells were harvested and expression level of the activation marker CD86 was analyzed by FACS.

In vivo functionality

Female hCD40tg mice of 8-14 weeks of age were inoculated with $10x10^6$ MC38-CEACAM5 cells s.c. in the right flank. On days 10, 13 and 16 after inoculation, the mice were administered i.p. with 167 µg of AC_05355 or vehicle control. The tumors were frequently measured with a Caliper instrument in regard to width (w), length (l) and height (h) and the tumor volume was calculated using the formula: (w/2 x l/2 x h/2 x π x (4/3)).

Toxicity study in cynomolgus monkeys

Toxicity testing in cynomolgus monkeys was conducted by Charles River Laboratories Edinburgh Ltd. Cynomolgus monkeys aged 2 to 4 years, weighing 3 to 6 kg, were given

the test item AC_05355 at days 1 and 8 via intravenous infusion into the tail vein according to the experimental design in Table 18.

Table 18. Experimental design for toxicity testing of AC_05355 in cynomolgus monkeys

Group No	Test Item	Dose Level (mg/kg/dose)	Dose Volume (mL/kg)	Dose Concentration (mg/mL)	No of Animals
1	AC_05355	10	7.5	1.33	1M + 1F
2	AC_05355	37.5	7.5	5	1M + 1F

Standard in-life assessment, including monitoring of body weight, body temperature and food consumption, was performed continuously during the study and until study termination and necroscopy at day 11. Samples were collected for clinical pathology assessments at days 1 (pre-treatment), 4, 8 (pre-treatment) and 11. Macroscopic and microscopic examination of an extensive list of tissues was performed post necroscopy.

Results

In vitro functionality

The data demonstrate that the tested CD40xCEACAM5 RUBY™ bsAb AC_05355 induces upregulation of CD86 on cynomolgus B cells in the presence of cynomolgus CEACAM5 expressed on CHO cells (*Figure 20*).

In vivo functionality

The data demonstrate that treatment with the CD40-CEACAM5 bsAb AC_05355 significantly reduces the MC38-CEACAM5 tumor volume compared to vehicle-treated mice at day 17 after tumor inoculation (*Figure 21*).

Toxicity study in cynomolgus monkeys

During the study duration, covering two weekly intravenous doses of AC_05355 at 10 and 37.5 mg/kg to male and female cynomolgus monkeys, no compound related adverse clinical signs were observed. Neither were any macroscopic finding observed post necroscopy, no deviating individual organ weight values obtained, and the microscopic findings observed were of the nature commonly observed in this strain and age of monkey, and, therefore, were considered not to be associated with the administration of AC_05355.

Cytokine levels in samples taken at 0, 4 and 24h post dosing at day 1 and 8 were in general low and no increases that could be reliably attributed to the dosing with AC_05355 were obtained (Table 18 to Table 23). Levels of IFN-γ, IL-2, IL-6 and IL-10 were below lower limit of quantification (LLOQ) at all time points for three out of four animals and only reached measurable levels at sporadic timepoints in a single monkey. Measurable levels of TNF-α were observed in the two male animals but not in any sampled from female animals (Table 23). However, the slight changes in TNF-α levels did not appear related to the dose level and therefore could not be reliably attributed to dosing with AC_05355.

Table 19. IFN-γ levels (pg/mL)

Group	Dose Level (mg/kg/dose)	Animal sex	Day 1			Day 8		
			0 h	4 h	24 h	0 h	4 h	24 h
1	10	M	43.4	53.4	<LLOQ	<LLOQ	63.1	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
2	37.5	M	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ

LLOQ = 37.5 pg/mL

Table 20. IL-2 levels (pg/mL)

Group	Dose Level (mg/kg/dose)	Animal sex	Day 1			Day 8		
			0 h	4 h	24 h	0 h	4 h	24 h
1	10	M	52.7	65.2	<LLOQ	<LLOQ	65.3	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
2	37.5	M	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ

LLOQ = 37.5 pg/mL

Table 21. IL-6 levels (pg/mL)

Group	Dose Level (mg/kg/dose)	Animal sex	Day 1			Day 8		
			0 h	4 h	24 h	0 h	4 h	24 h
1	10	M	<LLOQ	158	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
2	37.5	M	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ

LLOQ = 37.5 pg/mL

Table 22. IL-10 levels (pg/mL)

Group	Dose Level (mg/kg/dose)	Animal sex	Day 1			Day 8		
			0 h	4 h	24 h	0 h	4 h	24 h
1	10	M	193	230	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
2	37.5	M	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ

LLOQ = 188 pg/mL

Table 23. TNF-α levels (pg/mL)

Group	Dose Level (mg/kg/dose)	Animal sex	Day 1			Day 8		
			0 h	4 h	24 h	0 h	4 h	24 h
1	10	M	176	237	<LLOQ	<LLOQ	273	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ

2	37.5	M	<LLOQ	111	<LLOQ	<LLOQ	<LLOQ	<LLOQ
		F	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ

LLOQ = 37.5 pg/mL

Conclusion

Based on the above presented data, it can be concluded that the CD40xCEACAM5 RUBY™ bsAb AC_05355 is functional both *in vitro* and *in vivo*, with the ability to activate cynomolgus B cells in the presence of surface expressed cynomolgus CEACAM5. It can also be concluded that AC_05355 can be safely administered to cynomolgus monkey at two weekly doses up to at least 37.5mg/kg/dose, without provoking any adverse clinical signs, macro- or microscopic abnormalities or changes in cytokine levels that could be reliably attributed to the dosing with AC_05355.

Example 13

Materials and methods

MB49 CEA overexpressing cells were labeled with the fluorescent dye PKH26 (Sigma-Aldrich) according to manufacturer's instructions. Labeled MB49-CEA cells were heat-shocked at 45° C for 10 min to induce necrosis, followed by incubation at 37°C over night. The heat-shocked cells were then centrifuged and the supernatant containing necrotic tumor cell line debris was collected. Raji cells were labeled with the nuclear dye Hoechst 33342 (Thermo Scientific) and cultured with necrotic debris and titrated antibodies (ffAC_05337 or 1132). Images were captured using a Cytation 5 live cell imager (BioTek) and the number of PKH26-stained tumor debris co-localized with Hoechst-stained Raji cells was quantified using Gen5 software (BioTek).

Results

A dose-dependent increase in clusters of necrotic debris from a CEA-transfected MB49 tumor cell line with Raji cells was seen when the CD40xCEA targeting ffAC_05337 bsAb was added, but not with the monospecific CD40 Ab 1132 (as shown in Figure 24).

Example 14 - Functional assays using cells obtained from primary human colorectal cancer patients

Dissociated primary cells from colorectal cancer patients were purchased from Discovery Life Sciences (Huntsville, AL). Directly after thawing, DTCs were counted

using NucleoCounter® NC-200™ (Chemometec, Denmark) and 20,000 viable cells were pipetted into each well. The cancer cells were used to assess functionality in the CD40 bioassay, or alternatively the ability of the primary cancer cells to activate the immune cells in the same tumor sample was analyzed. 200,000 viable cells were pipetted into a Nunc UpCell 96-well plate (Thermo Scientific, 174897). Next, ATOR-4066 or controls were added into the wells. The plate was incubated for 48 hours in a 37°C, 5% CO2 incubator. Next, the cells were harvested, and analyzed by flow cytometry.

Table 24 – FACs panel for activation staining (22 tubes were stained)

CD40	APC	555591	BD Pharmingen
CD86	PE	555658	BD Pharmingen
CD45	V450	560367	BD Horizon
CD14	PerCP-Cy5.5	562692	BD Pharmingen
CD83	PE-Cy7	561132	BD Pharmingen
CD3	FITC	555332	BD Pharmingen
CD56	FITC	562794	BD Pharmingen

Results

First, it was demonstrated that the CEA densities in patient derived tumors were sufficient to provide cross-linking and induce CD40 stimulation using a reporter cell assay. The results demonstrated patient derived cancer cells can induce similar cross-linking and CD40 activation as the cell lines (Figure 25). Secondly, when culturing dissociated cells from patient derived colon tumors, it was demonstrated that a CD40xCEA bsAb (ffAC_05337) could activate tumor infiltrating immune cells (Figure 25).

Example 15 - Kinetic profiles of CD40xCEA and CD40xEpCAM RUBY™ bsAbs

Kinetic measurements were performed in the Octet RED96 platform with bispecific antibodies captured to anti human IgG Fc Capture (AHC) or FAB2G Biosensor tips (Sartorius). Monomeric human CD40-His-Avi tag (Acro Biosystems), monomeric human CEACAM5-His (R&D Systems) or monomeric human EpCAM-His (R&D Systems) were ½ serially diluted in 1x kinetic buffer (Sartorius) starting at 500nM or 100nM. Binding kinetics was studied in 1x kinetic buffer where association was allowed for 100 to 300 sec followed by dissociation for 100 to 3600 sec. Sensor tips were regenerated with 10mM Glycine pH 1.7. Data generated were referenced by subtracting blank or

parallel buffer blank, the baseline was aligned to the y-axis, inter-step correction by alignment against dissociation was performed and the data was smoothed by Savitzky-Golay filter in the data analysis software (v9.0.0.14). The processed data was fitted using a 1:1 Langmuir binding model.

Table 25

	CD40 affinity			TAA affinity		
bsAb ID	K_D (M)	k_{on} (1/Ms)	k_{off} (1/s)	K_D (M)	k_{on} (1/Ms)	k_{off} (1/s)
CD40xCEA #1	9.7E-08	4.5E+05	4.4E-02	1.8E-08	2.4E+05	4.3E-03
CD40xCEA #2	9.7E-08	4.5E+05	4.4E-02	2.1E-09	2.4E+05	4.2E-04
CD40xEpCAM #1	1.9E-06	1.1E+05	2.1E-01	4.8E-07	2.0E+04	1.0E-02
CD40xEpCAM #2	9.7E-08	4.5E+05	4.4E-02	4.8E-07	2.0E+04	1.0E-02

CD40xCEA #1 = ffAC_05337
CD40xCEA #2 = Multi46

Example 16

Materials and methods

Human samples
The collection of the tonsillar cancer sample at Lund University Hospital was approved by the Swedish Ethical Review Authority (ref. no. 2017/580), and the participating patient granted written informed consent.

Cell isolation and coculture
The tonsillar cancer biopsy was cut into small fragments in RPMI 1640 medium (ThermoFisher Scientific) supplemented with 0.1mg/mL gentamycin (Sigma-Aldrich). The tissue fragments were enzymatically digested with Collagenase IV (Sigma-Aldrich) (2.0 mg/mL) and DNase I (Sigma Aldrich) (200 Kunits/mL) for 20 minutes at 37° C. Cells were filtered using a 70 µm cell strainer (BD Biosciences) and stained with CD3-PerCPCy5.5, VS620-PECF594, CD45-APCH7 and HLA-DR-BV711 for cell sorting using FACSAria IIu (BD Biosciences). 104 viable CD45+ HLA-DR+ CD3- cells were sorted directly into 96-well flat-bottom plates (Nunc UpCell, ThermoFisher Scientific) pre-seeded with 6x104 UV-irradiated CHO-CEA cells, per well. 19nM of CD40xCEA bsAbs, CD40 mAbs or isotype controls were added to the cocultures for 13 h, after which the supernatants were collected, and the cells were washed and blocked for non-specific binding with ChromPure mouse IgG (Jackson ImmunoResearch) for 15 min at room

temperature. Cells were immediately stained with fluorochrome-coupled antibodies (Supplementary Table 1) for flow cytometric analysis using a FACSAria IIu instrument (BD Biosciences). Cytokine analysis was performed using Bio-Plex Pro Human Cytokine 17-plex Assay on the Bio-PlexR 200 system (Bio-Rad Lab).

Table 26 - Antibodies used for flow cytometry.

Antigen	Fluorophore	Clone	Company
Human cells			
CD45	APCH7	2D1	BD Biosciences
HLA-DR	BV711	G46-6	BD Biosciences
CD11c	BV510	B-ly6	BD Biosciences
XCR1	PE	S15046E	BioLegend
CD1C	BV786	L161	BioLegend
CD14	FITC	TÜK4	Invitrogen
CCR2	APC	K036C2	BioLegend
CD123	AF700	6H6	BioLegend
CD19	PerCP Cy5.5	HIB19	BD Biosciences
CD20	PerCP Cy5.5	2H7	BD Biosciences
CD86	BV605	IT2.2	BioLegend
CD40	PE-Cy7	5C3	BD Biosciences
CCR7	BV421	2-L1-A	BD Biosciences

Results

As shown in Figure 26, simultaneous binding of CD40 and CEA by CD40xCEA bsAbs (ffAC_05337) mediates activation of tonsillar cancer APCs *in vitro*.

Example 17 – tumour localisation

Materials and Methods

Human CD40 transgenic mice were inoculated with MC38-hCEA tumor cells (MC-38-CEA-2, Kerafast) s.c. and were administered with 100 µg anti-CD40 antibody or a molar equivalent dose (167 µg) CD40xCEA bsAb (ffAC_05337) or Isotype bsAb i.p. on days 10 and 13. On day 14, tumors were dissected. Frozen tumor sections were stained for human IgG to assess accumulation of administered antibodies, and for CEA to assess CEA expression pattern in the tumors.

Results

Figure 27 shows accumulation of the CD40xCEA bsAb (ffAC_05337), but not corresponding CD40 mAb, in CEA-expressing tumors

Example 18 – Immune status MB49

Materials and Methods

Cryo preserved tumors (B16.F10-hCD40+ # 6, 7 and 9 used as control, hereafter called B16 AND MB49 #2, 4 and 5) from human CD40 transgenic mice were analyzed. 8µm cryosections were prepared and stained. Mouse spleen was used as positive control. The sections were analyzed in a Leica DMRX-e microscope and representative photos were taken.

Results

Figure 28 shows that a marked higher degree of infiltrating T cells are seen in the MB49 tumors compared to the B16 tumors used as control.

Example 19 – Receptor density

The receptor density of CEA on particular cells was determined using a receptor density kit (Quantum Simply Cellular, anti-human IgG) according to manufacturer's instructions.

Table 27 – Receptor Density

Cell line	Celltype	CEA/cell
LOVO	Human	5,500
HT29	Human	11,300
LS174T	Human	51,500
MKN45	human	353,000
MC38-CEA1	Mouse (transfected)	300,000
CHO-CEA	Human (transfected)	125,000
Cell line	**Celltype**	**EpCAM/cell**
BxPC3	Human	260,000
JAR	Human	2,200,000
MCF7	Human	1,500,000
JEG3	human	2,400,000
MB49-EpCAM	Mouse (transfected)	230,000

CHO-EpCAM	Human (transfected)	350,000

Example 20 – Surrogate toxicology study

Materials and Methods

B cell activation of the cynoCEAxCD40 RUBY™ (AC_05355) on cynomolgus and human B cells in the presence of CEA transfected cells (macaque CEA, NP_001040590.1). Primary cynomolgus B cells were cultured with titrated antibodies in the presence CEA expressed on CHO cells. After 2 days, expression of CD86 on B cells was analyzed by FACS.

The cynoCEAxCD40 bispecific antibody (AC_05355) was administered once weekly via intravenous infusion for 2 weeks to cynomolgus monkeys at two different dose levels (10 mg/kg and 37.5 mg/kg). One female and one male were evaluated at each dose level.

Results

Figure 29 shows that CEAxCD40 bsAbs in the RUBY™ format (AC_05355) induce upregulation of CD86 on cynomolgus and human B cells to a similar degree. The CEA-conditional activation of CD40 on cynomolgus B cells and human B cells is similar to what is observed with the human CEAxCD40 bsAb in RUBY™ (AC_05355) used for the in vitro assays. The cynoCEAxCD40 bsAb binds with similar affinity to human and cynomolgus monkey CEA (hCEA vs cCEA, right panel).

Figure 29 also shows that in cynomolgus monkeys there were no findings associated with cyoCEAxCD40 bsAb (AC_05355) at the evaluated dose levels.

Example 21 – Receptor binding

Materials and Methods

Dual ELISA showing simultaneous binding of CD40xTAA RUBY™ bsAb to its respective antigen targets (CD40xCEA bsAb = ffAC_05337 and cynoCEAxCD40 bsAb = AC_05355). ELISA plates were coated with the target, bsAb was added followed by detection using biotinylated target.

Results

Figure 30 shows that the bispecific antibodies were successfully generated in the RUBY™format and the generated bsAbs displayed good binding to their respective antigen targets as illustrated by the ELISA binding evaluations.

Example 22: Anti-tumor effect of CD40-CEACAM5 bispecific antibody ffAC_05337

Background and aim

ffAC_05337 is a CD40-CEA bispecific antibody in the RUBY™ format. The antibody has been LALA-mutated to silence Fcγ receptor binding.

The aim of this study was to evaluate the anti-tumor effect of ffAC_05337 and a CD40 mAb in human CD40 transgenic (hCD40tg) mice inoculated with a CEACAM5-transfected murine tumor cell line called MC38-CEACAM5 2.

Materials and methods

Female hCD40tg mice of 9 weeks of age were inoculated with $1x10^6$ MC38-CEACAM5 2 cells (obtained from Kerafast) s.c. in the right flank. On days 7, 10, and 13 after inoculation, the mice were administered i.p. with 100 µg of wildtype CD40 monospecific antibody, 1132, or 167 µg of the CD40-CEACAM5 bsAb ffAC_05337. A group of vehicle-treated mice was also included. The tumors were frequently measured with a caliper in width (w), length (l) and height (h) and the tumor volume was calculated using the formula: (w/2 x l/2 x h/2 x π x (4/3)).

Results and conclusions

The data demonstrated that treatment with the CD40-CEACAM5 bsAb ffAC_05337 but not the CD40 mAb 1132 reduced the MC38-CEACAM5 tumor volume compared to vehicle-treated mice (Figure 31). Further, treatment with ffAC_05337 but not 1132 led to improved survival compared to vehicle-treated mice (Figure 31).

Example 23 - Functional assays using cells obtained from primary human gastric cancer patients

Dissociated primary cells from gastric cancer patients were purchased from Discovery Life Sciences (Huntsville, AL). Directly after thawing, DTCs were counted using NucleoCounter® NC-200™ (Chemometec, Denmark) and 20,000 viable cells were pipetted into each well. The cancer cells were used to assess functionality in the CD40 bioassay, or alternatively the ability of the primary cancer cells to activate the immune cells in the same tumor sample was analyzed. 200,000 viable cells were pipetted into a Nunc UpCell 96-well plate (Thermo Scientific, 174897). Next, CD40xCEA bsAb (ffAC_05337) or controls were added into the wells. The plate was incubated for 48 hours in a 37°C, 5% CO2 incubator. Next, the cells were harvested, and analyzed by flow cytometry.

Table 28 – FACs panel for activation staining

CD40	APC	555591	BD Pharmingen
CD86	PE	555658	BD Pharmingen
CD45	V450	560367	BD Horizon
CD14	PerCP-Cy5.5	562692	BD Pharmingen
CD83	PE-Cy7	561132	BD Pharmingen
CD3	FITC	555332	BD Pharmingen
CD56	FITC	562794	BD Pharmingen

Results

First, it was demonstrated that the CEA densities in patient derived tumors were sufficient to provide cross-linking and induce CD40 stimulation using a reporter cell assay. The results demonstrated that patient derived cancer cells can induce similar cross-linking and CD40 activation as the cell lines (Figure 32). Secondly, when culturing dissociated cells from patient derived gastric tumors, it was demonstrated that a CD40xCEA bsAb (ffAC_05337) could activate tumor infiltrating immune cells (Figure 32).

Example 24 – Evaluation of the CD40 agonistic function in the presence of soluble CEA using the CD40 reporter assay.

Aim and background

The aim of this study was to assess the CD40 agonistic function of the bispecific antibody ffAC_05337 using the CD40 reporter assay in the presence of CEA expressing cells and physiological relevant soluble CEA concentrations. CD40 crosslinking will be mediated by simultaneous binding of CD40, expressed on CD40 reporter cells, and CEA

expressed on CHO cells or CEA expressing human tumor cells. In addition, since high levels of soluble CEA can be detected systemically in cancer patients, the agonistic function was assessed in the presence of physiological relevant concentrations of soluble CEA.

Materials and methods

Agonistic function of the ffAC_05337 was evaluated using a CD40 reporter assay (Promega, CD40 Bioassay Kit CS JA2155). The assay was performed according to the manufacturer's protocol. In brief, CD40 reporter cells and titrating concentrations of ffAC_05337 were diluted in RPMI containing 10% FCS and added to the assay plates before the addition of CEA transfected CHO, CHO wt or CEA expressing human tumor cells. In addition, the assay was performed in the presence of 0.5, 1, 5, 25 or 50 ug/ml soluble CEA. The assay plates were incubated for 6 h at 37°C until addition of Bio-GloTM Luciferase Assay Detection solution and analyzed in the BMG ELISA plate reader.

Results and conclusions

The results show that the bispecific antibody ffAC_05337 induce CD40 activation in the presence of CEA and the potency and efficacy is unaffected by the presence of soluble CEA in the cultures (Figure 33 and 34). A minor decrease in the efficacy of ffAC_05337 can be observed in the presence of 25 and 50 ug/ml soluble CEA (Figure 34).

Claims

1. A bispecific polypeptide comprising a first binding domain, designated B1, which is capable of binding specifically to CD40, and a second binding domain, designated B2, which is capable of specifically binding to carcinoembryonic antigen (CEA).

2. The bispecific polypeptide according to Claim 1, wherein the first and/or second binding domains are/is selected from the group consisting of antibodies and antigen-binding fragments thereof, and CD40 ligands.

3. The bispecific polypeptide according to Claim 2 wherein the antigen-binding fragment is selected from the group consisting of: Fv fragments (such as a single chain Fv fragment, or a disulphide-bonded Fv fragment), Fab-like fragments (such as a Fab fragment; a Fab' fragment or a F(ab)2 fragment) and domain antibodies.

4. The bispecific polypeptide according to any one of the preceding claims wherein the polypeptide is a bispecific antibody.

5. The bispecific polypeptide according to any one of the preceding claims wherein:

(a) binding domain B1 and/or binding domain B2 is an intact IgG antibody;
(b) binding domain B1 and/or binding domain B2 is an Fv fragment;
(c) binding domain B1 and/or binding domain B2 is a Fab fragment; and/or
(d) binding domain B1 and/or binding domain B2 is a single domain antibody.

6. The bispecific polypeptide according to Claim 5, wherein the bispecific antibody comprises a human Fc region or a variant of a said region, where the region is an IgG1, IgG2, IgG3 or IgG4 region, preferably an IgG1 or IgG4 region.

7. The bispecific polypeptide according to Claim 6, wherein the Fc exhibits no or very low affinity for FcγR.

8. The bispecific polypeptide according to Claim 7, wherein the Fc region is a variant of a human IgG1 Fc region comprising a mutation at one or more of the following positions:

L234, L235, P239, D265, N297 and/or P329.

9. The bispecific polypeptide according to Claim 8, wherein alanine is present at the mutated position(s).

10. The bispecific polypeptide according to Claim 9, wherein the Fc region is a variant of a human IgG1 Fc region comprising the double mutations L234A and L235A.

11. The bispecific polypeptide according to any one of Claims 4-10, wherein the bispecific antibody is selected from the groups consisting of:

(a) bivalent bispecific antibodies, such as IgG-scFv bispecific antibodies (for example, wherein B1 is an intact IgG and B2 is an scFv attached to B1 at the N-terminus of a light chain and/or at the C-terminus of a light chain and/or at the N-terminus of a heavy chain and/or at the C-terminus of a heavy chain of the IgG, or vice versa);
(b) monovalent bispecific antibodies, such as a DuoBody® or a 'knob-in-hole' bispecific antibody (for example, an scFv-KIH, scFv-KIHr, a BiTE-KIH or a BiTE- KIHr);
(c) scFv2-Fc bispecific antibodies (for example, ADAPTIR™ bispecific antibodies);
(d) BiTE/scFv2 bispecific antibodies;
(e) DVD-Ig bispecific antibodies;
(f) DART-based bispecific antibodies (for example, DART2-Fc or DART);
(g) DNL-Fab3 bispecific antibodies; and
(h) scFv-HSA-scFv bispecific antibodies.

12. The bispecific polypeptide according to Claim 11, wherein the bispecific antibody is an IgG-scFv bispecific antibody.

13. The bispecific polypeptide according to any one of the preceding claims, wherein binding domain B1 and binding domain B2 are fused directly to each other.

14. The bispecific polypeptide according to any one of the preceding claims, wherein binding domain B1 and binding domain B2 are joined via a polypeptide linker.

15. The bispecific polypeptide according to Claim 14, wherein the linker is selected from the group consisting of the amino acid sequence SGGGGSGGGGS (SEQ ID NO: 337), SGGGGSGGGGSAP (SEQ ID NO: 338), NFSQP (SEQ ID NO: 339), KRTVA (SEQ ID NO: 340), GGGSGGGG (SEQ ID NO: 341), GGGGSGGGGS (SEQ ID NO: 342), GGGGSGGGGSGGGGS (SEQ ID NO: 343), GSTSGSGKPGSGEGSTKG (SEQ ID NO: 344),

THTCPPCPEPKSSDK (SEQ ID NO: 345), GGGS (SEQ ID NO: 346), EAAKEAAKGGGGS (SEQ ID NO: 347), EAAKEAAK (SEQ ID NO: 348), or (SG)m, where m = 1 to 7.

16. The bispecific polypeptide according to any one of the preceding claims, wherein one of B1 or B2 is an immunoglobulin molecule, and one of B1 or B2 is a Fab fragment, wherein the Fab fragment is fused to the C-terminus of the heavy chain of the immunoglobulin via the light chain of the Fab fragment.

17. The bispecific polypeptide according to any one of the preceding claims, wherein the bispecific polypeptide comprises one or more mutations to promote association of the heavy chain polypeptide of the immunoglobulin with the light chain polypeptide of the immunoglobulin and/or to promote association of the heavy chain polypeptide of the Fab with the light chain polypeptide of the Fab.

18. The bispecific polypeptide according to Claim 17, wherein the one or more mutations prevent the formation of aggregates and a Fab by-product.

19. The bispecific polypeptide according to Claim 17 or 18, wherein the mutations prevent formation of aggregates and Fab by-products by generating steric hindrance and/or incompatibility between charges.

20. The bispecific polypeptide according to any one of Claims 17-19, wherein the polypeptide comprises one or more mutation pairs each comprising two functionally compatible mutations.

21. The bispecific polypeptide according to any one of the preceding claims, wherein the polypeptide can modulate the activity of and/or activate myeloid cells.

22. The bispecific polypeptide according to any one of the preceding claims, wherein the polypeptide is incapable of inducing antibody-dependent cell cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) and/or complement-dependent cytotoxicity (CDC).

23. The bispecific polypeptide according to any one of the preceding claims, wherein the polypeptide is capable of inducing tumour immunity.

24. The bispecific polypeptide according to any one of the preceding claims, wherein the polypeptide is capable of inducing:

(a) tumour-specific immune activation; and/or

(b) activation of dendritic cells; and/or

(c) internalisation of associated tumour debris and/or extracellular vesicles containing CEA antigens as well as tumour neoantigens; and/or

(d) cross-presentation of peptides derived from internalised tumour antigens on MHC; and/or

(e) priming and activation of effector T cells; and/or

(f) direct tumoricidal effects, selected from the list consisting of: apoptosis, necroptosis, antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC).

25. The bispecific polypeptide according to any one of the preceding claims, wherein the bispecific polypeptide is capable of:

(a) activation of a B-cell, in the presence of a CEA; and/or

(b) activation of dendritic cells in the presence of CEA; and/or

(c) capable of increased dendritic cell cross-presentation of neoantigens; and/or

(d) inducing proliferation of neoantigen specific T cells.

26. The bispecific polypeptide according to any one of the preceding claims, wherein the bispecific polypeptide promotes uptake of tumor derived material, derived from tumor cells overexpressing CEA.

27. The bispecific polypeptide according to any one of the preceding claims, wherein the B-cell activation is characterised by CD86 upregulation.

28. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B1 binds to human CD40 with a K_D of less than $2x10^{-7}$M or less than $1.5x10^{-7}$M or less than $8.5x10^{-8}$M or less than $8x10^{-8}$M or less than $7.5x10^{-8}$M or less than $7x10^{-8}$M or less than $9x10^{-8}$M or less than $9x10^{-9}$M or less than $5x10^{-10}$M or less than $3x10^{-10}$M, preferably less than $8.5x10^{-8}$M, more preferably less than $5x10^{-10}$M or less than $3x10^{-10}$M.

29. The bispecific polypeptide according to any one of the preceding claims, wherein binding domain B1 comprises one or more heavy chain CDR sequences selected from those in Table C(1) and/or wherein binding domain B1 comprises one or more light chain CDR sequences selected from those in Table C(2).

30. The bispecific polypeptide according to any one of the preceding claims, wherein binding domain B1 comprises one, two or three light chain CDR sequences from a particular row for an individual antibody reference in Table C(2), and/or one, two or three heavy chain CDR sequences from the corresponding row for the antibody with the same reference in Table C(1).

31. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B1 comprises all three heavy chain CDR sequences of a particular antibody reference as shown in Table C(1), and/or all three light chain CDR sequences of an antibody reference as shown in Table C(2), or wherein binding domain B1 comprises a heavy chain VH sequence and/or a light chain VL sequence as shown in Table A.

32. The bispecific polypeptide according to any one of the preceding claims, wherein B1 comprises any one, two, three, four, five or all six features independently selected from the following:

(a) a heavy chain CDR1 sequence which consists of the sequence "G, F, T, F, S, S, Y, A";

(b) a heavy chain CDR2 sequence which is 8 amino acids in length and comprises the consensus sequence: "I, G/S, S/G, Y/S, G/S, G/S, G/Y/S, T";

(c) a heavy chain CDR3 sequence which is 9 to 12 amino acids in length and which comprises the consensus sequence of : "A, R, Y/R/G, Y/P/V/-, N/S/V, F/Y/W, G/H/S, -/S, -/V, M/F, D, Y"

(d) a light chain CDR1 sequence which consists of the sequence: "Q, S, I, S, S, Y";

(e) a light chain CDR2 sequence which consists of the sequence: "A, A, S";

(f) a light chain CDR3 sequence which is 9 amino acids in length and comprises the consensus sequence: "Q, Q, Y/S, G/Y, R/S/V, N/A/Y/T, P, P/F/Y, T".

33. A bispecific polypeptide according to any one of the preceding claims wherein binding domain B1 comprises:

(a) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1132/1133 (SEQ ID NOs: 73, 74 and 75; and/or 90, 91 and 92); or

(b) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1107/1108 (SEQ ID NOs: 73, 78 and 80; and/or SEQ ID NOs: 90, 91 and 95); or

(c) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1150/1151 (SEQ ID NOs: 73, 76 and 77; and/or SEQ ID NOs: 90, 91 and 93); or

(d) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 1140/1135 (SEQ ID NOs: 73, 78 and 79; and/or SEQ ID NOs: 90, 91 and 94); or

(e) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody G12 or G12_mut or ffAC_05337 (SEQ ID NOs: 81, 82 and 83; and/or SEQ ID NOs: 96, 97, and 98); or

(f) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody APX005 (SEQ ID NOs: 84, 85 and 86; and/or SEQ ID NOs: 99, 100, and 101); or

(g) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody 21.4.1 (SEQ ID NOs: 87, 88 and 89; and/or SEQ ID NOs: 102, 103, and 104).

34. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B1 comprises:

(a) the heavy chain variable region and/or the light chain variable region of antibody 1132/1133 (SEQ ID NOs: 3 and 1); or

(b) the heavy chain variable region and/or the light chain variable region of antibody 1107/1108 (SEQ ID NOs: 15 and 13); or

(c) the heavy chain variable region and/or the light chain variable region of antibody 1150/1151 (SEQ ID NOs: 7 and 5); or

(d) the heavy chain variable region and/or the light chain variable region of antibody 1140/1135 (SEQ ID NOs: 11 and 9); or

(e) the heavy chain variable region and/or the light chain variable region of antibody G12 (SEQ ID NOs: 19 and 17); or

(f) the heavy chain variable region and/or the light chain variable region of antibody APX005 (SEQ ID NOs: 23 and 21); or

(g) the heavy chain variable region and/or the light chain variable region of antibody 21.4.1 (SEQ ID NOs: 27 and 25); or

(h) the heavy chain variable region and/or the light chain variable region of antibody G12_mut (SEQ ID NOs: 29 and 17); or

(i) the heavy chain variable region and/or the light chain variable region of antibody ffAC_05337 (SEQ ID NOs: 431 and 430).

35. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B1 comprises the light chain of antibody 1132/1133 (SEQ ID NO: 372 or 379) and/or the heavy chain of antibody 1132/1133 (SEQ ID NO: 371 or 378).

36. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B1 comprises the light chain of antibody G12 (SEQ ID NO: 381) and/or the heavy chain of antibody G12 (SEQ ID NO: 380) or the light chain of antibody G12_mut (SEQ ID NO: 383) and/or the heavy chain of antibody G12_mut (SEQ ID NO: 382).

37. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B1 comprises the light chain of antibody ffAC_05337 (SEQ ID NO: 430) and/or the heavy chain of antibody ffAC_05337 (SEQ ID NO: 431).

38. The bispecific polypeptide according to any one of the preceding claims, wherein the CEA is a tumor-associated CEA.

39. The bispecific polypeptide according to any one of the preceding claims, wherein the CEA is a carcinoembryonic antigen-related cell adhesion molecule (CEACAM).

40. The bispecific polypeptide according to Claim 39, wherein the CEACAM is one or more selected from the listing consisting of: CEACAM1; CEACAM6; and CEACAM5.

41. The bispecific polypeptide according to Claim 40, wherein the CEACAM is CEACAM5.

42. The bispecific polypeptide according to any one of the preceding claims, wherein B2 which is capable of specifically binding to CEA on a target cell.

43. The bispecific polypeptide according to Claim 42, wherein the target cell is a cancer cell and/or a tumour cell.

44. The bispecific polypeptide according to Claim 43, wherein the CEA on the target cell is an intermediate level of CEA or a high level of CEA.

45. The bispecific polypeptide according to Claim 44, wherein the intermediate level of CEA is characterised by the target cell expressing about 10,000 or more CEA receptors per target cell, preferably about 50,000 or more CEA receptors per target cell.

46. The bispecific polypeptide according to Claim 44, wherein the high level of CEA is characterised by the target cell expressing about 200,000 of more CEA receptors per target cell, preferably about 300,000 of more CEA receptors per target cell.

47. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B2 binds to human CEA with a K_D of less than $2x10^{-6}$M or less than $1.5x10^{-8}$M or less than $2.5x10^{-9}$M or less than $2x10^{-9}$M or less than $1.5x10^{-12}$M or less than $1x10^{-12}$M, preferably less than $1.5x10^{-8}$M or less than $2.5x10^{-9}$M or less than $1.5x10^{-12}$M.

48. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B2 binds preferentially to CEA on a cell over soluble CEA.

49. The bispecific polypeptide according to any one of the preceding claims, wherein binding domain B2 comprises one or more heavy chain CDR sequences selected from those in Table D(1a) and/or Table D(1b) and/or wherein binding domain B2 comprises one or more light chain CDR sequences selected from those in Table D(2).

50. The bispecific polypeptide according to any one of the preceding claims, wherein binding domain B2 comprises one, two or three light chain CDR sequences from a particular row for an individual antibody reference in Table D(2), and/or one, two or three heavy chain CDR sequences from the corresponding row for the antibody with the same reference in Table D(1a) and/or Table D(1b).

51. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B2 comprises all three heavy chain CDR sequences of a particular antibody reference as shown in Table D(1a) and/or Table D(1b), and/or all three light chain CDR sequences of an antibody reference as shown in Table D(2), or wherein binding domain B1 comprises a heavy chain VH sequence and/or a light chain VL sequence as shown in Table B.

52. The bispecific polypeptide according to any one of the preceding claims, wherein B2 comprises any one, two, three, four, five or all six features independently selected from the following:

(a) a heavy chain CDR1 sequence which consists of the sequence: "G, F, T, F, S, S, S, Y" or which comprises the consensus sequence of: "G, F, T, F, G/S, S, Y, Y/A";

(b) a heavy chain CDR2 sequence which consists of the sequence: "I, G, S, G, S, Y, S, T" or which comprises the consensus sequence of: "I, S, G, Y/S, G, Y/G, S, T";

(c) a heavy chain CDR3 sequence which comprises the consensus sequence of: "A, R, Y, P, S, V, P/L, F, P, Q, S, P/H/L, H/P/L, L/F/V/W, D, Y" or which comprises the consensus sequence of: "A, R, H/Y, G, Y, G/S/T, V/ H, L/F, D, Y";

(d) a light chain CDR1 sequence which consists of the sequence: "Q, S, I, S, S, Y" or which comprises the consensus sequence of: "Q, S, I, R/S, S, Y";

(e) a light chain CDR2 sequence which consists of the sequence: "A, A, S";

(f) a light chain CDR3 sequence which consists of the sequence: "Q, Q, A, G, N, P, H, T" or which comprises the consensus sequence of: "Q, Q, G/Y, T/P/A, W/-, Y/-, F/V, P, F/Y, T".

53. A bispecific polypeptide according to any one of the preceding claims wherein binding domain B2 comprises:

(a) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05059 (SEQ ID NOs: 216, 217 and 218 or 280, 281 and 218 and/or SEQ ID NOs: 90, 91 and 311)

(b) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05060 (SEQ ID NOs: 219, 220 and 221 or 282, 283 and 221 and/or SEQ ID NOs: 312, 91 and 313)

(c) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05061 (SEQ ID NOs: 222, 223 and 224 or 284, 285 and 224 and/or SEQ ID NOs: 90, 91 and 314)

(d) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05062 (SEQ ID NOs: 222, 223 and 225 or 284, 285 and 225 and/or SEQ ID NOs: 315, 316 and 94)

(e) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05064 (SEQ ID NOs: 222, 223 and 226 or 284, 285 and 226 and/or SEQ ID NOs: 90, 91 and 317)

(f) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05079 (SEQ ID NOs: 216, 217 and 227 or 280, 281 and 227 and/or SEQ ID NOs: 90, 91 and 311)

(g) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05080 (SEQ ID NOs: 216, 217 and 228 or 280, 281 and 228 and/or SEQ ID NOs: 90, 91 and 311)

(h) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05081 (SEQ ID NOs: 216, 217 and 229 or 280, 281 and 229 and/or SEQ ID NOs: 90, 91 and 311)

(i) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05082 (SEQ ID NOs: 222, 223 and 230 or 284, 285 and 230 and/or SEQ ID NOs: 90, 91 and 311)

(j) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05083 (SEQ ID NOs: 222, 223 and 231 or 284, 285 and 231 and/or SEQ ID NOs: 318, 91 and 319)

(k) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05084 (SEQ ID NOs: 222, 223 and 232 or 284, 285 and 232 and/or SEQ ID NOs: 90, 91 and 320)

(l) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05085 (SEQ ID NOs: 219, 233 and 234 or 286, 287 and 234 and/or SEQ ID NOs: 90, 91 and 311)

(m) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05086 (SEQ ID NOs: 216, 217 and 235 or 280, 281 and 235 and/or SEQ ID NOs: 90, 91 and 311)

(n) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05087 (SEQ ID NOs: 216, 217 and 236 or 280, 281 and 236 and/or SEQ ID NOs: 90, 91 and 311)

(o) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05088 (SEQ ID NOs: 216, 217 and 237 or 280, 281 and 237 and/or SEQ ID NOs: 90, 91 and 311)

(p) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05089 (SEQ ID NOs: 216, 217 and 238 or 280, 281 and 238 and/or SEQ ID NOs: 90, 91 and 311)

(q) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05090 or ffAC_05337 (SEQ ID NOs: 216, 217 and 239 or 280, 281 and 239 and/or SEQ ID NOs: 90, 91 and 311)

(r) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05091 (SEQ ID NOs: 216, 217 and 240 or 280, 281 and 240 and/or SEQ ID NOs: 90, 91 and 311)

(s) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05092 (SEQ ID NOs: 216, 217 and 218 or 280, 281 and 218 and/or SEQ ID NOs: 321, 91 and 311)

(t) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05093 (SEQ ID NOs: 216, 217 and 241 or 280, 281 and 241 and/or SEQ ID NOs: 90, 91 and 311)

(u) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05094 (SEQ ID NOs: 216, 217 and 242 or 280, 281 and 242 and/or SEQ ID NOs: 90, 91 and 311)

(v) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05095 (SEQ ID NOs: 216, 217 and 243 or 280, 281 and 243 and/or SEQ ID NOs: 90, 91 and 311)

(w) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05096 (SEQ ID NOs: 216, 217 and 244 or 280, 281 and 244 and/or SEQ ID NOs: 90, 91 and 311)

(x) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05097 (SEQ ID NOs: 217, 216 and 245 or 280, 281 and 245 and/or SEQ ID NOs: 90, 91 and 311)

(y) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05098 (SEQ ID NOs: 219, 220 and 246 or 282, 283 and 246 and/or SEQ ID NOs: 312, 91 and 313)

(z) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05099 (SEQ ID NOs: 222, 223 and 224 or 288, 285 and 224 and/or SEQ ID NOs: 90, 91 and 311)

(aa) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody AC_05100 (SEQ ID NOs: 222, 223 and 247 or 288, 285 and 247 and/or SEQ ID NOs: 90, 91 and 311)

(ab) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab1 (SEQ ID NOs: 248, 249 and 250 or 289, 290 and 250 and/or SEQ ID NOs: 90, 91 and 322)

(ac) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab2 (SEQ ID NOs: 251, 252 and 253 or 291, 292 and 253 and/or SEQ ID NOs: 90, 91 and 323)

(ad) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab3 (SEQ ID NOs: 254, 255 and 256 or 293, 294 and 256 and/or SEQ ID NOs: 324, 325 and 326)

(ae) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab4 (SEQ ID NOs: 257, 258 and 259 or 295, 296 and 259 and/or SEQ ID NOs: 90, 91 and 327)

(af) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab5 (SEQ ID NOs: 260, 261 and 262 or 297, 298 and 262 and/or SEQ ID NOs: 324, 325 and 328)

(ag) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab6 (SEQ ID NOs: 263, 264 and 265 or 299, 300 and 265 and/or SEQ ID NOs: 324, 325 and 329)

(ah) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab7 (SEQ ID NOs: 266, 267 and 268 or 301, 302 and 268 and/or SEQ ID NOs: 90, 91 and 330)

(ai) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab8 (SEQ ID NOs: 269, 270 and 271 or 303, 304 and 271 and/or SEQ ID NOs: 90, 91 and 331)

(aj) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab9 (SEQ ID NOs: 272, 335 and 273 or 305, 306 and 273 and/or SEQ ID NOs: 90, 91 and 332)

(ak) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab10 (SEQ ID NOs: 274, 275 and 276 or 307, 308 and 276 and/or SEQ ID NOs: 90, 91 and 333) and/or

(al) the three CDRs of the heavy chain and/or the three CDRs of the light chain of antibody Fab11 (SEQ ID NOs: 277, 278 and 279 or 309, 310 and 279 and/or SEQ ID NOs: 324, 325 and 334).

54. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B2 comprises:

(a) the heavy chain variable region and/or the light chain variable region of antibody AC_05059 (SEQ ID NO: 33 and/or SEQ ID NO: 31)

(b) the heavy chain variable region and/or the light chain variable region of antibody AC_05060 (SEQ ID NO: 37 and/or SEQ ID NO: 35)

(c) the heavy chain variable region and/or the light chain variable region of antibody AC_05061 (SEQ ID NO: 41 and/or SEQ ID NO: 39)

(d) the heavy chain variable region and/or the light chain variable region of antibody AC_05062 (SEQ ID NO: 45 and/or SEQ ID NO: 43)

(e) the heavy chain variable region and/or the light chain variable region of antibody AC_05064 (SEQ ID NO: 49 and/or SEQ ID NO: 47)

(f) the heavy chain variable region and/or the light chain variable region of antibody AC_05079 (SEQ ID NO: 53 and/or SEQ ID NO: 51)

(g) the heavy chain variable region and/or the light chain variable region of antibody AC_05080 (SEQ ID NO: 57 and/or SEQ ID NO: 55)

(h) the heavy chain variable region and/or the light chain variable region of antibody AC_05081 (SEQ ID NO: 61 and/or SEQ ID NO: 59)

(i) the heavy chain variable region and/or the light chain variable region of antibody AC_05082 (SEQ ID NO: 65 and/or SEQ ID NO: 63)

(j) the heavy chain variable region and/or the light chain variable region of antibody AC_05083 (SEQ ID NO: 69 and/or SEQ ID NO: 67)

(k) the heavy chain variable region and/or the light chain variable region of antibody AC_05084 (SEQ ID NO: 106 and/or SEQ ID NO: 71)

(l) the heavy chain variable region and/or the light chain variable region of antibody AC_05085 (SEQ ID NO: 110 and/or SEQ ID NO: 108)

(m) the heavy chain variable region and/or the light chain variable region of antibody AC_05086 (SEQ ID NO: 114 and/or SEQ ID NO: 112)

(n) the heavy chain variable region and/or the light chain variable region of antibody AC_05087 (SEQ ID NO: 118 and/or SEQ ID NO: 116)

(o) the heavy chain variable region and/or the light chain variable region of antibody AC_05088 (SEQ ID NO: 122 and/or SEQ ID NO: 120)

(p) the heavy chain variable region and/or the light chain variable region of antibody AC_05089 (SEQ ID NO: 126 and/or SEQ ID NO: 124)

(q) the heavy chain variable region and/or the light chain variable region of antibody AC_05090 (SEQ ID NO: 130 and/or SEQ ID NO: 128)

(r) the heavy chain variable region and/or the light chain variable region of antibody AC_05091 (SEQ ID NO: 134 and/or SEQ ID NO: 132)

(s) the heavy chain variable region and/or the light chain variable region of antibody AC_05092 (SEQ ID NO: 138 and/or SEQ ID NO: 136)

(t) the heavy chain variable region and/or the light chain variable region of antibody AC_05093 (SEQ ID NO: 142 and/or SEQ ID NO: 140)

(u) the heavy chain variable region and/or the light chain variable region of antibody AC_05094 (SEQ ID NO: 146 and/or SEQ ID NO: 144)

(v) the heavy chain variable region and/or the light chain variable region of antibody AC_05095 (SEQ ID NO: 150 and/or SEQ ID NO: 148)

(w) the heavy chain variable region and/or the light chain variable region of antibody AC_05096 (SEQ ID NO: 154 and/or SEQ ID NO: 152)

(x) the heavy chain variable region and/or the light chain variable region of antibody AC_05097 (SEQ ID NO: 158 and/or SEQ ID NO: 156)

(y) the heavy chain variable region and/or the light chain variable region of antibody AC_05098 (SEQ ID NO: 162 and/or SEQ ID NO: 160)

(z) the heavy chain variable region and/or the light chain variable region of antibody AC_05099 (SEQ ID NO: 166 and/or SEQ ID NO: 164)

(aa) the heavy chain variable region and/or the light chain variable region of antibody AC_05100 (SEQ ID NO: 170 and/or SEQ ID NO: 168)

(ab) the heavy chain variable region and/or the light chain variable region of antibody Fab1 (SEQ ID NO: 174 and/or SEQ ID NO: 172)

(ac) the heavy chain variable region and/or the light chain variable region of antibody Fab2 (SEQ ID NO: 178 and/or SEQ ID NO: 176)

(ad) the heavy chain variable region and/or the light chain variable region of antibody Fab3 (SEQ ID NO: 182 and/or SEQ ID NO: 180)

(ae) the heavy chain variable region and/or the light chain variable region of antibody Fab4 (SEQ ID NO: 186 and/or SEQ ID NO: 184)

(af) the heavy chain variable region and/or the light chain variable region of antibody Fab5 (SEQ ID NO: 190 and/or SEQ ID NO: 188)

(ag) the heavy chain variable region and/or the light chain variable region of antibody Fab6 (SEQ ID NO: 194 and/or SEQ ID NO: 192)

(ah) the heavy chain variable region and/or the light chain variable region of antibody Fab7 (SEQ ID NO: 198 and/or SEQ ID NO: 196)

(ai) the heavy chain variable region and/or the light chain variable region of antibody Fab8 (SEQ ID NO: 202 and/or SEQ ID NO: 200)

(aj) the heavy chain variable region and/or the light chain variable region of antibody Fab9 (SEQ ID NO: 206 and/or SEQ ID NO: 204)

(ak) the heavy chain variable region and/or the light chain variable region of antibody Fab10 (SEQ ID NO: 210 and/or SEQ ID NO: 208)

(al) the heavy chain variable region and/or the light chain variable region of antibody Fab11 (SEQ ID NO: 214 and/or SEQ ID NO: 212)

(am) the heavy chain variable region and/or the light chain variable region of antibody mAb2 (SEQ ID NO: 387 and /or SEQ ID NO: 385) and/or

(an) the heavy chain variable region and/or the light chain variable region of antibody ffAC_05337 (SEQ ID NO: 433 and /or SEQ ID NO: 432).

55. The bispecific polypeptide according to any one of the preceding claims wherein binding domain B2 comprises:

(a) the light chain and/or the heavy chain of antibody AC_05059 (SEQ ID NO: 388 and/or SEQ ID NO: 389)

(b) the light chain and/or the heavy chain of antibody AC_05060 (SEQ ID NO: 390 and/or SEQ ID NO: 391)

(c) the light chain and/or the heavy chain of antibody AC_05061 (SEQ ID NO: 392 and/or (SEQ ID NO: 393)

(d) the light chain and/or the heavy chain of antibody AC_05062 (SEQ ID NO: 394 and/or SEQ ID NO: 395)

(e) the light chain and/or the heavy chain of antibody AC_05064 (SEQ ID NO: 396 and/or SEQ ID NO: 397)

(f) the light chain and/or the heavy chain of antibody AC_05079 (SEQ ID NO: 398 and/or SEQ ID NO: 399)

(g) the light chain and/or the heavy chain of antibody AC_05081 (SEQ ID NO: 400 and/or SEQ ID NO: 401)

(h) the light chain and/or the heavy chain of antibody AC_05088 (SEQ ID NO: 402 and/or SEQ ID NO: 403)

(i) the light chain and/or the heavy chain of antibody AC_05089 (SEQ ID NO: 404 and/or SEQ ID NO: 405)

(j) the light chain and/or the heavy chain of antibody AC_05090 (SEQ ID NO: 406 and/or SEQ ID NO: 407)

(k) the light chain and/or the heavy chain of antibody AC_05091 (SEQ ID NO: 408 and/or SEQ ID NO: 409)

(l) the light chain and/or the heavy chain of antibody AC_05093 (SEQ ID NO: 410 and/or SEQ ID NO: 411)

(m) the light chain and/or the heavy chain of antibody AC_05094 (SEQ ID NO: 412 and/or SEQ ID NO: 413)

(n) the light chain and/or the heavy chain of antibody AC_05096 (SEQ ID NO: 414 and/or SEQ ID NO: 415)

(o) the light chain and/or the heavy chain of antibody AC_05097 (SEQ ID NO: 416 and/or SEQ ID NO: 417)

(p) the light chain and/or the heavy chain of antibody Fab1 (SEQ ID NO: 418 and/or SEQ ID NO: 419)

(q) the light chain and/or the heavy chain of antibody Fab3 (SEQ ID NO: 420 and/or (SEQ ID NO: 421).

56. The bispecific polypeptide according to any one of the preceding claims wherein the bispecific polypeptide:

- comprises a Chain H1 comprising a sequence selected from the listing consisting of: SEQ ID NO: 359; SEQ ID NO: 362; SEQ ID NO: 365; and/or SEQ ID NO: 367; and/or
- comprises a Chain L1 comprising a sequence selected from the listing consisting of: SEQ ID NO: 360; SEQ ID NO: 363; SEQ ID NO: 372; and/or SEQ ID NO: 368; and/or
- comprises a Chain H2 comprising a sequence selected from the listing consisting of: SEQ ID NO: 361; SEQ ID NO: 364; SEQ ID NO: 366; and/or SEQ ID NO: 369.

57. A polypeptide comprising a binding domain, designated B2, as defined in any one of Claims 1 to 56, which is capable of specifically binding to CEA.

58. A bispecific polypeptide comprising a first binding domain, designated B3, which is capable of binding specifically to a target antigen that is not CD40, and a second binding domain, designated B2, as defined in any of Claims 1 to 57, which is capable of specifically binding to CEA.

59. An isolated nucleic acid molecule encoding a bispecific polypeptide according to any one of Claims 1-56 or 58 or a polypeptide according to Claim 57, or a component polypeptide chain thereof.

60. The nucleic acid molecule according to Claim 59 wherein the molecule is a cDNA molecule.

61. The nucleic acid molecule according to Claim 59 or 60 encoding an antibody heavy chain or variable region thereof.

62. The nucleic acid molecule according to any one of Claims 59 to 61 encoding an antibody light chain or variable region thereof.

63. The vector comprising a nucleic acid molecule according to any one of Claims 59 to 62.

64. The vector according to Claim 63 wherein the vector is an expression vector.

65. A recombinant host cell comprising a nucleic acid molecule according to any one of Claims 59 to 62 or a vector according to Claim 63 or 64.

66. The host cell according to Claim 65 wherein the host cell is a bacterial cell.

67. The host cell according to Claim 65 wherein the host cell is a mammalian cell.

68. The host cell according to Claim 67 wherein the host cell is a human cell.

69. A method for producing bispecific polypeptide according to any one of Claims 1-56 or 58 or a polypeptide according to Claim 57, the method comprising culturing a

host cell as defined in any one of Claims 65 to 68 under conditions which permit expression of the bispecific polypeptide or component polypeptide chain thereof.

70. A pharmaceutical composition comprising an effective amount of bispecific polypeptide according to any one of Claims 1-56 or 58 or an effective amount of a polypeptide according to Claim 59, and a pharmaceutically-acceptable diluent, carrier or excipient.

71. The pharmaceutical composition according to Claim 70 adapted for parenteral delivery.

72. The pharmaceutical composition according to Claim 70 adapted for intravenous delivery.

73. A bispecific polypeptide according to any one of Claims 1-56 or 58 or a polypeptide according to Claim 57, for use in medicine.

74. A bispecific polypeptide according to any one of Claims 1-56 or 58 or a polypeptide according to Claim 57, for use in treating cancer and/or a tumour and/or a non-cancer condition in a subject.

75. Use of a bispecific polypeptide according to any one of Claims 1-56 or 58 or a polypeptide according to Claim 57, in the preparation of a medicament for treating a cancer and/or a tumour and/or a non-cancer condition in a subject.

76. The bispecific polypeptide or polypeptide for use according to Claim 74 or the use according to Claim 75, wherein the polypeptide is for use in combination with one or more additional therapeutic agents.

77. The bispecific polypeptide or polypeptide for use according to Claim 76 or the use according to Claim 76, wherein the one or more additional therapeutic agents is/are an immunotherapeutic agent that binds a target selected from the group consisting of PD-1/PD-L1, VGFR, EGFR, HER2, CTLA-4, CD137, OX40, GITR, LAG3, TIM3, CD27, VISTA and KIR.

78. The bispecific polypeptide or polypeptide for use according to any one of Claims 74, 76 or 77 or the use according to any one of Claims 75-77, for administration systemically.

79. A method for the treatment and/or diagnosis of a cancer and/or a tumour and/or a non-cancer condition in a subject, comprising the step of administering to the subject an effective amount of a bispecific polypeptide according to any one of Claims 1-56 or 58 or a polypeptide according to Claim 57.

80. A method according to Claim 79, wherein the method comprises administering the bispecific polypeptide systemically or the polypeptide systemically.

81. A method according to Claim 79 or 80, further comprising administering to the subject one or more additional therapeutic agents.

82. A method according to Claim 81, wherein the one or more additional therapeutic agents is/are an immunotherapeutic agent that binds a target selected from the group consisting of PD-1/PD-L1, VGFR, EGFR, HER2, CTLA-4, CD137, OX40, GITR, LAG3, TIM3, CD27 and KIR.

83. The bispecific polypeptide or polypeptide for use according to any one of Claims 74 or 76-78, the use according to any one of Claims 75-78, or the method according to any one of Claims 80-83, wherein the cancer and/or the tumour comprises target cells comprising expression of CEA.

84. The bispecific polypeptide or polypeptide for use according to Claim 83, the use according to Claim 83, or the method according to Claim 83, wherein the expression of CEA is an intermediate level of CEA expression or a high level of CEA expression.

85. The bispecific polypeptide or polypeptide for use according to Claim 84, the use according to Claim 84, or the method according to Claim 84, wherein the intermediate level of CEA expression is characterised by the target cell expressing about 10,000 or more CEA receptors per target cell, preferably about 50,000 or more CEA receptors per target cell.

86. The bispecific polypeptide or polypeptide for use according to Claim 84, the use according to Claim 84, or the method according to Claim 84, wherein the high level of CEA expression is characterised by the target cell expressing about 200,000 of more CEA receptors per target cell, preferably about 300,000 of more CEA receptors per target cell.

87. The bispecific polypeptide or polypeptide for use according to any one of Claims 74 or 76-86, the use according to any one of Claims 75-86, or the method according to any one of Claims 79-86, wherein the cancer and/or the tumour is one or more cancer and/or tumour selected from the list consisting of: prostate cancer and/or a prostate tumour; breast cancer and/or a breast tumour; lung cancer and/or a lung tumour; colorectal cancer and/or a colorectal tumour; melanomas; bladder cancer and/or a bladder tumour; brain/CNS cancer and/or a brain/CNS tumour; cervical cancer and/or a cervical tumour; oesophageal cancer and/or a oesophageal tumour; gastric cancer and/or a gastric tumour; head/neck cancer and/or a head/neck tumour; kidney cancer and/or a kidney tumour; liver cancer and/or a liver tumour; carcinoma; leukaemia; lymphomas; ovarian cancer and/or an ovarian tumour; pancreatic cancer and/or a pancreatic tumour; tonsil cancer and/or a tonsil tumour; and sarcomas.

88. The bispecific polypeptide or polypeptide for use according to Claim 87, the use according to Claim 87, or the method according to Claim 87, wherein the one or more cancer and/or tumour selected from the list consisting of: breast cancer and/or a breast tumour; lung cancer and/or a lung tumour; colorectal cancer and/or a colorectal tumour; gastric cancer and/or a gastric tumour; and/or pancreatic cancer and/or a pancreatic tumour.

89. The bispecific polypeptide or polypeptide for use according to any one of Claims 74 or 76-88, the use according to any one of Claims 75-88, or the method according to any one of Claims 79-88, wherein the tumour is a solid tumour.

90. The bispecific polypeptide or polypeptide for use according to any one of Claims 74 or 76-78, the use according to any one of Claims 75-78, or the method according to any one of Claims 79-82, wherein the one or more non-cancer condition is selected from the list consisting of: ulcerative colitis, pancreatitis; cirrhosis; COPD; Crohn's disease; and/or hypothyroidism.

91. The bispecific polypeptide or polypeptide for use according to any one of Claims 74 or 76-90, the use according to any one of Claims 75-90, or the method according to any one of Claims 79-90, wherein the subject is human.

92. A bispecific polypeptide substantially as described herein with reference to the description and figures.

93. A polypeptide substantially as described herein with reference to the description and figures.

94. A polynucleotide substantially as described herein with reference to the description and figures.

95. A pharmaceutical composition substantially as described herein with reference to the description and figures.

96. A bispecific polypeptide for use or a polypeptide for use, substantially as described herein with reference to the description and figures.

97. Use of a bispecific polypeptide substantially as described herein with reference to the description and figures.

98. A method of treatment substantially as described herein with reference to the description and figures.