BISPECIFIC ANTIBODIES AGAINST CEACAM5 AND CD3

FIELD OF THE INVENTION

The present invention relates to bispecific antibodies which bind to human carcinoembryonic antigen CEACAM5 (CEA) and human CD3ε (CEAxCD3 bispecific antibody). In addition, the present invention relates to polynucleotides encoding such bispecific antibodies and vectors and host cells comprising such polynucleotides. The invention further relates to methods for selecting and producing such antibodies and to methods of using such antibodies in the treatment of diseases.

BACKGROUND OF THE INVENTION

Successful treatment of advanced/metastatic solid cancer like e.g. pancreatic cancer, colorectal cancer stomach cancer, lung cancer etc. is still a challenge. Modern cancer immunotherapy has introduced methods/techniques to help the body`s immune cells to better attack and kill cancer cells. For example, several techniques/methods have been developed to increase the attack of tumor cells by T-cells. Examples are immune checkpoint inhibitors like e.g. PD-1/PD-L1 inhibiting monoclonal antibodies, T-cell bispecific antibodies binding to a tumor associated antigen (TAA) and CD3 on T-cells or CAR-T-cells. CAR-T-cells and bispecific antibodies are effective in hematological malignancies and approved for e.g. the treatment of B-cell malignancies or acute lymphocytic leukemia ALL, but so far there has been no real breakthrough of these methods in the therapy of advanced/metastatic solid cancer. Monoclonal antibodies and also bispecific antibodies used in therapy can cause a variety of adverse effects. An important toxicity issue is the cytokine-release syndrome (CRS), which was for example found in therapy with alemtuzumab, muromonab-CD3, rituximab, tosituzumab and CD19xCD3 bispecific antibody Blinatumomab.

Tabernero et.al. (J Clin Oncol 35, 2017 (suppl. abstr. 3002)) presented at ASCO 2017 phase 1 clinical data in patients with advanced/metastatic colorectal cancer with an CEAxCD3 bispecific antibody (RO 6958688, cibisatamab, see below) in monotherapy and in combination with the anti-PD-L1 antibody atezolizumab. Cibisatamab has a so called 2+1 format, with one Fab fragment binding to CD3 and with two Fab fragments binding to CEA. Such antibodies are e.g. described in US20140242079 (WO2014131712) and US20140242080 (WO2014131711).

As used herein “TCB2014 “refers to a bispecific antibody binding to CEA and CD3 in the 2+1 format as described in US20140242080 (incorporated by reference in its entirety), comprising as CDRs the CDRs as shown in SEQ ID NOs: 270-276 and 290-296 of US20140242080 (see also CDRs of SEQ ID NOs: 4-10 and 24-30 of US20140242079, incorporated by reference in its entirety). As used herein “TCB2017” refers to molecule B in the "2+1 IgG CrossFab, inverted" format with charge modifications (VH/VL exchange in CD3 binder, charge modification in CEA binder, humanized CEA binder) as described in WO2017055389 (incorporated by reference in its entirety) comprising as CDRs the CDRs as shown in SEQ ID NOs: 4-6, 8-10 and 14-19 of WO2017055389.

The 2+1 structure is rather different from native IgG antibodies. The structure also contains artificial amino acid (aa) bridges and two different heavy chains brought together by knob into hole technology/aa sequences in the Fc part (see e.g. US6737056, WO2013055958). Such bispecific antibodies (e.g., RO6958688, cibisatamab) are immunogenic and cause therefore formation of anti-drug-antibodies (ADA) and loss of drug exposure due to the neutralization of the drug by ADA. Melero et al reported at 60-200 mg doses 50 or more % of patients with ADA and in 45% of patients loss of exposure (Melero et al., ASCO 2017, Abstract 2549 and Poster No. 41, Abstract see Journal of Clinical Oncology 35, no. 15_suppl (May 20, 2017) 2549-2549). Loss of exposure makes practical therapy difficult to control and significantly decreases the probability for success. To minimize ADA formation, cibisatamab respectively the combination of cibisatamab and atezolizumab is tested clinically in combination after pre-treatment with the anti-CD20 antibody obinutuzumab (see ClinicalTrials.gov Trial NCT03866239). The pre-treatment is given to deplete the B-cells of the patients with metastatic colorectal carcinomas. Depletion of B-cells leads to a drop of immunoglobulins of the patient and therefore of potential ADAs, but at the same time it leads to a weakening of the immune system.

MEDI-565 (AMG211), a further bispecific CEAxCD3 antibody, a single-chain antibody, has been in clinical development, results have been published (see e.g. M. Pishvaian et al. Clin Colorectal Cancer. 2016 DEC; 15(4) 345-351). The study NCT01284231 (ClinicalTrials.gov) is reported as completed, no new trial has been started in the last years. This single chain bispecific antibody (two scFv connected by an aa linker) has extremely low elimination half-life between

2.2 and 6.5 hours (Pishvaian et al.; Clin. Colorectal Cancer, 2016 DEC; 15(4) 345-351) (herein incorporated by reference).

The present invention provides a less immunogenic CEAxCD3 bispecific antibody with high efficacy. Such an antibody comprises a common heavy chain and in one embodiment a kappa light chain in the CEA binding part and a lambda light chain in the CD3 binding part.

The concept of using a common heavy chain for obtaining bispecific antibodies is mentioned in Fischer et al., Nature Communications 6 (2015): 6113. https://doi.org/10.1038/ncomms7113 and Magistrelli G. et al., MABS 9 (2017) 231-239. Kappa lambda bispecific antibodies are described in e.g. WO2014087248 (hereby incorporated by reference in its entirety). Their structure is almost indistinguishable from the structure of a native IgG with the consequence of no or minimal ADA formation and thus low or minimal loss of exposure. The sequence of the common heavy chain variable region VH and the sequence huCD3 VL 1A4 of the invention are described in WO2019175658 (US2019/0284297) (incorporated by reference herein in its entirety).

As mentioned above, WO2017055389 describes bispecific CEAxCD3 antibodies with the 2+1 format but binding to a different domain as cibisatamab. One of these antibodies (RO7172508 or RG 6123) has been tested in a clinical trial in patients with locally advanced and/or metastatic CEA- positive solid tumors (ClinicalTrials.gov; search for RO7172508), also with obinutuzumab pre-treatment and in combination with atezolizumab. According to the description of the clinical trial in ClinicalTrials.gov for some cohorts serum CEA (shed soluble CEACAM5, sCEA) levels below a certain threshold were required in patients to be treated to make them eligible to the treatment, suggesting that higher levels of shed soluble CEACAM5 can decrease the efficacy of this CEAxCD3 bispecific antibody. The antibodies of the current invention show minimal influence of shed soluble CEA on their tumor cell killing efficacy.

Shed soluble CEACAM5 is an established tumor marker. Levels of sCEA in plasma of cancer patients can go over 1000 ng/ml whereas plasma concentrations in healthy individuals are below 10 ng/ml (e.g. Sandler B. et al Anticancer Res 1999, 19(5B), 4229-33). Shed soluble CEACAM5 can therefore compete with membrane-bound CEA present on tumor cells for the binding of therapeutic anti-CEA antibodies and anti-CEA bispecific antibodies, potentially causing

decreased efficacy of an anti-CEA antibody or a CEAxCD3 antibody. TCB2017 and TCB2014 (see above) have been tested by the inventors in vitro in a test for T-cell mediated lysis of CEA positive tumor cells in the presence of soluble CEA. It was found that addition of sCEA to the test shifts the lysis curve and therefore the EC50 value of TCB2014 and TCB2017 to higher concentrations, suggesting that both TCB2014 and TCB2017 bind significantly to sCEA.

The human CEA family contains 29 genes, of which 18 are expressed: 7 belong to the CEA subgroup and 11 to the pregnancy-specific glycoprotein subgroup. Several CEA subgroup members are thought to possess cell adhesion properties. CEACAM5 is not only expressed by colorectal cancer cells but also by pancreatic cancer, stomach cancer, lung cancer and other cancer types. CEACAM5 is thought to have a role in innate immunity (Hammarström S., Semin. Cancer Biol. 9(2):67-81 (1999)). Carcinoembryonic antigen 5 (CEA, CEACAM5 or CD66e; UniProtKB - P06731) is a member of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM family) and a tumor-associated antigen (Gold and Freedman, J Exp. Med., 121:439-462, 1965; Berinstein N. L., J Clin Oncol., 20:2197-2207, 2002). Multiple monoclonal antibodies have been raised against CEACAM5 for research purposes, as diagnostic tools, and for therapeutic purposes (see e.g. WO2012117002). Members of the carcinoembryonic antigen family (CEACAMs) are widely expressed, and, depending on the tissue, capable of regulating diverse functions including tumor promotion, tumor suppression, angiogenesis, and neutrophil activation. Four members of this family, CEACAM1, CEACAM3, CEACAM6, and CEACAM8 are expressed and enriched on human neutrophils (http://www.proteinatlas.org). Given the mechanism of action of CEAxCD3 bispecific antibodies, cross-reactivity with other CEACAM could lead to depletion of important circulating healthy cell populations. E.g. cross-reactivity with CEACAM8, which is expressed by neutrophils or hematopoietic stem cells, could lead to the depletion of such cell populations. The invention provides a CEAxCD3 bispecific antibody with low cross reactivity to one or more of the members of the CEACAM family, CEACAM1, CEACAM3, CEACAM4, CEACAM6, CEACAM7, CEACAM8, CEACAM16, CEACAM18, CEACAM19, CEACAM20, and CEACAM21.

The mouse monoclonal anti-CEACAM5 antibody PR1A3 was raised by fusion of NS1 (P3/NS l/I-Ag-4-l) myeloma cells with spleen cells from mice immunized with normal colorectal epithelium. Richman P. I. and Bodmer W. F., Int. J. Cancer, 39:317-328, 1987 describe mouse

monoclonal antibody PR1A3. Epitope mapping of PRl A3 shows that the antibody targets the B3 domain and the GPI anchor of the CEA molecule (Durbin H. et al., Proc. Natl. Acad. Sci. USA, 91 :4313-4317, 1994). The epitope bound by PRl A3 is a conformational epitope, not a linear epitope (Stewart et al., Cancer Immunol. Immunother., 47 (1999) 299-06). Humanized PRlA3 (hPRlA3) antibodies are described e.g. by Conaghhan P. J., et al., Br. J. Cancer, 98 (2008)1217-1225 and WO2012117002. The CEA binder used in TCB2014 (named CH1A1A) is a humanized, affinity matured, and stability-engineered version derived from the PR1A3 antibody. M. Bacac et.al., Clin. Cancer Research 22(13);3286-97 (2016), Conaghan P, et al., Br J Cancer 2008;98:1217–25, and Durbin H, et al. Proc Natl Acad Sci U S A 1994;91:4313–7).

A method for treating cancer by a combination of a human PD-1 axis antagonist and a bispecific anti-CEAxCD3 antibody is mentioned in WO2017118657 and clinical results have been published at ASCO conference 2017 (Tabernero et al, J Clin Oncol 35, 2017 (suppl. abstr. 3002)). A method of treating tumors by administering immune checkpoint antagonists binding two or more different targets of an immune checkpoint pathway, and a T cell-redirecting agent binding to CEA and a T cell surface antigen is mentioned in WO2015112534. A conjugate consisting of a single domain anti-CEACAM6 antibody and urease is at present in clinical trials (NCT02309892; WO2016116907). A class I antibody binding to CEACAM5, CEACAM6 and granulocytes is mentioned in US20110064653. Bispecific antibodies comprising a first polypeptide chain and a second polypeptide chain, covalently bonded to one another are mentioned in WO2018053328.

An anti-CD3ε antibody described in the state of the art is SP34 (Yang SJ, The Journal of Immunology (1986) 137; 1097-1100). SP34 reacts with both primate and human CD3. SP34 is available from BD Biosciences. A further anti CD3 antibody described in the state of the art is UCHT-1 (see WO2000041474). A further anti CD3 antibody described in the state of the art is BC-3 (Fred Hutchinson Cancer Research Institute; used in Phase I/II trials of GvHD, Anasetti et al., Transplantation 54: 844 (1992)). SP34 differs from UCHT-1 and BC-3 in that SP-34 recognizes an epitope present on solely the ε chain of CD3 (see Salmeron et al., (1991) J. Immunol. 147: 3047) whereas UCHT-1 and BC-3 recognize an epitope contributed by both the ε and γ chains. Anti CD3 antibodies are also described in WO2007042261, WO2008119565, WO2008119566, WO2008119567, WO2010037836, WO2010037837, WO2010037838, and

US8236308. Bispecific antibodies comprising a binding part specific for CEA and a binding part specific for CD3ε are e.g. described in US20140242079, WO2007071426, WO2013012414, WO2015112534, WO2017118675, and WO2017055389. An anti CD3 antibody comprising sequences of the second binding part of an antibody according to the invention are mentioned in US62/643,095 and PCT/US2019/000232, incorporated herein by reference in its entirety. US2012321626 mentions a multi-specific Fab fusion protein comprising a Fab fragment that binds to the N-terminus of CD3 epsilon. WO2018199593 mentions bispecific antibodies that bind to HER3 and CD3.

As mentioned already above, results of first clinical trials with T-cell bispecific antibodies TAA x CD3 (TAA = Tumor Associated Antigen) in patients with advanced solid tumors were disappointing, but recently preliminary phase 1 results have been published for the CEAxCD3 bispecific antibody cibisatamab (RO6958688, see for example Bacac et al Clin. Cancer Res., 22(13), 3286-97 (2016); and US20140242079) showing in advanced colorectal cancer patients in monotherapy and in combination with PD-L1 inhibition partial responses and stable disease (J.Tabernero et.al., J. Clin. Oncol. 35, 2017 (suppl. Abstr. 3002)). Another approach to get better results could be to add to T-cell bispecific antibodies not only an inhibitor of PD-1 checkpoint axis, but to add further checkpoint inhibitors or agonists. But so far, it is believed there are no promising clinical data for such a combination approach available.

Limited availability of T-cells within advanced solid tumors is certainly an important mechanism limiting the efficacy achievable with T-cell bispecific antibodies plus PD-1 axis inhibitors.

Instead of adding to the combination of a T-cell bispecific antibody and a PD-1 axis inhibitor another therapeutic agent aiming to re-direct T-cells against tumor cells of advanced solid tumors, it may be more successful to add a therapeutic agent re-directing to the tumor cells other immune cells, especially macrophages or macrophages and natural killer (NK)-cells.

The present invention provides new CEAxCD3 bispecific antibodies which are designed in a way that they can be administered in parallel with CEAxCD47 bispecific antibodies re-directing macrophages and also NK-cells against CEA expressing solid tumors. The combined attack of T-

cells and macrophages and NK-cells targeted to CEA expressing tumors offers a considerable opportunity for superior efficacy/killing and phagocytosis of CEA expressing tumor cells.

The so far disappointing results with CAR T-cells in solid tumors may have a simple explanation – the number of CAR T-cells penetrating the solid tumor and distributed in it are just not sufficient. This is certainly different in the majority of haematological malignancies; CAR T-cells can well access the tumor cells, explaining the difference of high efficacy in these malignancies compared to disappointing efficacy in solid tumors. In addition, CAR T-cells may be heavily suppressed by the tumor microenvironment (TME) of the solid tumors which is mostly strongly immune suppressive.

The present invention provides novel bispecific anti-CEAxCD3 antibodies with high efficacy, low influence of sCEA on efficacy, low or no crossreactivity to other CEACAM than CEACAM5 (= CEA) and therefore decreased toxicity, low immunogenicity, the opportunity of parallel combination therapy with CEAxCD47 antibodies and with valuable pharmacokinetic properties.

SUMMARY

In one example, the disclosure refers to a bispecific antibody (further named also as "bsAb CEAxCD3" or "CEAxCD3 bispecific antibody") comprising a first binding part, specifically binding to human CEACAM5 (further named also as "CEA") and a second binding part, specifically binding to human CD3ε (further named also as "CD3").

In one example the bispecific antibody is characterized in that said antibody is monovalent for the first binding part and monovalent for the second binding part.

In one example the bispecific antibody is characterized in that the constant and variable framework region sequences are human.

In one example the bispecific antibody is characterized in that each of the first and second binding parts comprises an immunoglobulin heavy chain and an immunoglobulin light chain.

In one example the bispecific antibody has a first binding part comprising a heavy chain and a second binding part comprising a heavy chain wherein the heavy chain in each binding part is the same (i.e. a common heavy chain). In one example the common heavy chain variable region comprises as CDRs CDRH1 of SEQ ID NO: 2, CDRH2 of SEQ ID NO: 3, and CDRH3 of SEQ ID NO: 4. In one example the common heavy chain variable region is of SEQ ID NO: 1. In one example the common constant heavy chain is of SEQ ID NO: 30. In one example the common heavy chain is of SEQ ID NO: 43. In one example the common heavy chain is of SEQ ID NO: 44. In one example the common heavy chain is of SEQ ID NO: 45.

In one example the bispecific antibody is characterized in comprising in the first binding part and the second binding part as heavy chain a common heavy chain, comprising in the first binding part as light chain a kappa light chain, and in the second binding part as light chain a lambda light chain. In one example the light chain of the second binding part is of SEQ ID NO: 28 and the heavy chain of the second binding part is of SEQ ID NO: 45 (e.g. AB1 and AB-1L3-1/N derived bispecific antibodies like AB13L3-1/N, AB14L3-1/N, AB15L3-1/N, AB17L3-1/N, AB20L3-1/N, AB54L3-1/N, AB60L3-1/N, AB66L3-1N, AB71L3-1/N, AB72L3-1/N, and AB73L3-1/N; for CDR and VL sequences see sequence list).

AB13,14,15 etc. denote for a first binding part (anti-CEACAM5 antibody arms) and L3-1 denotes for a second binding part (anti-CD3 antibody arm, also called 1A4) of bispecific antibodies of this invention. Any ABXX anti CEA arm can be combined with the L3-1 anti CD3 arm to form a bispecific antibody: e.g. ABXXL3-1 denotes a CEAxCD3 bispecific antibody according to the invention, comprising a WT hIgG1 Fc part; ABXXL3-1/D denotes a CEAxCD3 bispecific antibody according to the invention, comprising a hIgG1 Fc part carrying the L234A + L235A mutations; ABXXL3-1/N denotes a CEAxCD3 bispecific antibody according to the invention, comprising a hIgG1 Fc part carrying the L234A + L235A + P329A mutations.

In one example the bispecific antibody is characterized in comprising in the first binding part and the second binding part as heavy chain a common heavy chain, comprising in the first binding part as light chain variable region a lambda type region and as light chain constant region

a kappa type region ("hybrid format light chain"), and in the second binding part as light chain a lambda light chain (e.g. L3-1AB8 H-CK5 /D see figure 2 and description of figure 2).

In one example the bispecific antibody is characterized in comprising in the first binding part and the second binding part as heavy chain a common heavy chain, comprising in the first binding part as light chain variable region a lambda type region and as light chain constant region a lambda type region, and in the second binding part as light chain variable region a lambda type region and as light chain constant region a kappa type region ("hybrid format light chain"); e.g. AB8L3-1 H-CK5/D.

The bispecific antibodies disclosed herein show low binding/cross-reactivity to CEACAM family members other than CEACAM5. In one example the bispecific antibody is characterized in that the MFI value for binding to PEAKrapid cells (ATCC® CRL-2828™) expressing a CEACAM selected from the group consisting of CEACAM1, CEACAM3, CEACAM4, CEACAM6, CEACAM7, CEACAM8, CEACAM16, CEACAM18, CEACAM19, CEACAM20, and CEACAM21 is no more than 2 times compared to the MFI value for binding to WT PEAK cells (that is, untransfected PEAK cells) under the same experimental conditions. In one example the bispecific antibody is characterized in that the MFI value for binding to PEAKrapid cells expressing a CEACAM selected form the group consisting of CEACAM1, CEACAM3, CEACAM4, CEACAM6, and CEACAM8 is no more than 2 times compared to the MFI value for binding to WT PEAK cells under the same experimental conditions. In one example the bispecific antibody is characterized in that the MFI value for binding to PEAKrapid cells expressing CEACAM8 is no more than 2 times compared to the MFI value for binding to WT PEAK cells under the same experimental conditions. The experimental procedure for transfection of the PEAK cells and measuring of the binding of antibodies to these PEAK cells is described in Examples 1 and 5.

In one example the bispecific antibody binds to MKN-45 cells (DSMZ No: ACC 409) with an EC50 value of 0.5 nM to 50 nM. In one example the bispecific antibody binds to MKN-45 cells with an EC50 value of 0.5 nM to 30 nM. In one example the bispecific antibody is characterized in that the MFI value for binding to MKN-45 cells at 200 nM, 1000 nM and 5000 nM is at least double of the MFI value obtained with TCB2014. The

binding assay is described in Example 7a. In one example the EC50 for the killing of tumor cell line MKN-45 measured in the assay containing human PBMC is for the bispecific antibodies of this invention 40% or more lower than the EC50 measured for TCB2014. In one example the EC50 for the killing of tumor cell line LS-174T measured in the assay containing human PBMC is for the bispecific antibodies disclosed herein 40% or more lower than the EC50 measured for TCB2014.

In one example the bispecific antibody is killing LS174T cells in an assay containing human PBMC in a concentration dependent manner with an EC50 value of 0.01 to 10 nM. In one example the bispecific antibody is killing LS174T cells in an assay containing human PBMC in a concentration dependent manner with an EC50 value of 0.01 to 1 nM.

An assay for measuring T-cell retargeted lysis/killing of CEA-positive cells is described in Example 8.

In one example the bispecific antibody is characterized in that the EC50 value in the same assay (killing of CEA positive LS174T tumor cells) is not increased by more than a factor of 20, in one example not more than 15, and in one example not more than 10, in presence of 5 µg/ml soluble CEACAM5, compared to the EC50 value for lysis without soluble CEACAM5 under the same experimental conditions.

In another example the bispecific antibody is characterized in that the EC50 value in the same assay is not increased by more than a factor of 10, in one examplenot more than 5, in presence of 1 µg/ml soluble CEACAM5, compared to the EC50 value for lysis without soluble CEACAM5 under the same experimental conditions.

In one example the bispecific antibody is characterized in that said bispecific antibody inhibits tumor volume growth in a HPAF-II model until day 18 by 25% or more, compared to tumor volume growth in the vehicle group under the same experimental conditions. In one example the bispecific antibody is characterized in that said bispecific antibody inhibits tumor volume growth in a HPAF-II model until day 18 similar, and not different in a statistically

significant manner, compared to TCB2014 under the same experimental conditions. The mouse tumor model is described in Example 9a.

In one example the bispecific antibody comprises in each subunit of the Fc domain amino acid substitutions that reduce binding to an activating Fc receptor and/or reduce effector function wherein said amino acid substitutions are L234A and L235A and/or a substitution of P329, selected from the group consisting of P329A, P329G and P329R (Kabat EU index numbering). In one example the bispecific antibody comprises in each subunit of the Fc domain amino acid substitutions L234A and L235A and P329A (Kabat EU index numbering). L234A and L235A (LALA) means that amino acid leucine at position 234/235 is replaced by alanine. P329A (PA) means that amino acid proline at position 329 is replaced by alanine.

In one example the bispecific antibody comprises a common heavy chain. In one example the bispecific antibody comprises a common heavy chain comprising as CDRs CDRH1 of SEQ ID NO: 2, as CDRH2 of SEQ ID NO: 3 and as CDRH3 of SEQ ID NO: 4. In one example the bispecific antibody comprises in the second binding part a light chain region comprising as CDRs CDRL1 of SEQ ID NO: 18, CDRL2 of SEQ ID NO: 19, and CDRL3 of SEQ ID NO: 20.

In one example the bispecific antibody comprises in the first binding part as light chain constant region a region of SEQ ID NO: 39. In one example the bispecific antibody comprises in the first binding part as light chain constant region a region of SEQ ID NO: 41. In one example the bispecific antibody comprises in the first binding part as light chain constant region a region of SEQ ID NO: 58. In one example the bispecific antibody comprises a common heavy chain of SEQ ID NO: 43 or a common heavy chain of SEQ ID NO: 44 or a common heavy chain of SEQ ID NO: 45. In one example the bispecific antibody comprises in the first binding part as light chain constant region a region of SEQ ID NO: 39 and a common heavy chain of SEQ ID of SEQ ID NO: 45.

In one example the bispecific antibody comprises in the second binding part as light chain a light chain selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, and 29 or from the hybrid format light chain (LC) group of SEQ ID NOs: 67, 68, 69, 70, and 71.

In one example the bispecific antibody comprises in the first binding part as light chain constant region a region of SEQ ID NO: 39, a common heavy chain of SEQ ID NO: 45, and in the second binding part a light chain of SEQ ID NO: 28.

In one example the bispecific antibody competes with an anti-CEA antibody, selected from the group consisting of an anti-CEA antibody, comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 48 and 49 (anti-CEA antibody MEDI), comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 46 and 47 (antibody SM3E), comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 56 and 57 (Labetuzumab (Lab)), comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 50 and 51 (SAR), comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 54 and 55 (T86.66), and comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 52 and 53 (CH1A1A). See also figure 1 and Example 5c).

Examples for antibodies useful as CEA VL or CL regions in bispecific antibodies according to the invention and competing with MEDI for the binding to recombinant CEA are anti-CEA antibody AB1 and antibodies obtained by lead optimizing of AB1 (see Example 11 for the experimental method). Anti-CEA antibodies AB13, 14, 15, 17, 20, 54, 60, 66, 71, 72, 73 and the respective bispecific anti CEAxCD3 antibodies AB13L3-1, AB14L3-1, AB15L3-1, AB17L3-1, AB20L3-1, AB54L3-1, AB60L3-1, AB66L3-1, AB71L3-1, AB72L3-1, and AB73L3-1 are competing in the same manner as antibody AB1 rsp. AB1L3-1. Examples for antibodies useful as CEA VL or CL regions in bispecific antibodies according to the invention and competing with SM3E for the binding to recombinant CEA are anti-CEA antibody AB8 and antibodies obtained by oligonucleotide-directed mutagenesis of AB8, using degenerated oligonucleotides. Examples for antibodies useful as CEA VL or CL regions in bispecific antibodies according to the invention and competing with T84.66 for the binding to recombinant CEA are anti-CEA antibody 1B4 and antibodies obtained by oligonucleotide-directed mutagenesis of 1B4, using degenerated oligonucleotides.

Examples for antibodies useful as CEA VL or CL regions in bispecific antibodies according to the invention but not competing with any of the reference antibodies for the binding to

recombinant CEA are anti-CEA antibody C11 and antibodies obtained by oligonucleotide-directed mutagenesis of C11, using degenerated oligonucleotides.

AB1 is an anti-CEA antibody with the HC SEQ ID NO: 43 and the kappa LC SEQ ID NO: 40, encoded by the nucleic acid sequences shown in SEQ ID NOs: 80 and 78, respectively.

AB8 is an anti-CEA antibody with the HC SEQ ID NO: 43 and the lambda LC SEQ ID NO: 42, encoded by the nucleic acid sequences shown in SEQ ID NOs: 80 and 79, respectively.

1B4 is an anti-CEA antibody with the HC SEQ ID NO: 43 and the lambda LC SEQ ID NO: 74, encoded by the nucleic acid sequences shown in SEQ ID NO: 80 and 77, respectively.

C11 is an anti-CEA antibody with the HC SEQ ID NO: 43 and the kappa LC SEQ ID NO: 73, encoded by the nucleic acid sequences shown in SEQ ID NO: 80 and 76, respectively.

A CEA light chain useful as kappa light chain is of SEQ ID NO: 40. A CEA light chain useful as kappa light chain is of SEQ ID NO: 73. A CEA light chain useful as lambda light chain is of SEQ ID NO: 74. A CEA light chain useful as hybrid kappa light chain is of SEQ ID NO: 75.

In one example the bispecific antibody comprises in each subunit of the Fc domain up to three amino acid substitutions that reduce binding to an activating Fc receptor and/or effector function wherein said amino acid substitutions are L234A, L235A and a substitution of P329, selected from the group consisting of P329A, P329G and P329R (Kabat EU index numbering). In one example the common heavy chain of the antibody disclosed herein is of SEQ ID NOs: 43, 44, or 45. In one example the common heavy chain of the antibody according to the disclosure is of SEQ ID NO: 45 (L234A, L235A, and P329A).

In one example the bispecific antibody shows one or more properties selected from the group of a) binding to MKN-45 cells with an EC50 value of 0.5 nM to 50 nM, b1) competing with an anti-CEA antibody, comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 48 and 49 (MEDI), or b2) competing with an anti-CEA antibody, comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 46 and 47 (SM3E), or b3) competing with an

anti-CEA antibody, comprising as VL and VH domains VL and CH of sequences SEQ ID NOs: 54 and 55 (T84.66), or b4) not competing with any of the tool antibodies (see Example 5c for the tool antibodies) c) comprising in each subunit of the Fc domain amino acid substitutions that reduce binding to an activating Fc receptor and/or effector function wherein said amino acid substitutions are L234A and L235A and a substitution of P329, selected from the group consisting of P329A, P329G and P329R (Kabat EU index numbering), d) killing MKN-45, HPAF-II, and/or LS174T cells in an assay containing human PBMC in a concentration dependent manner with an EC50 value of 0.01 to 10 nM.

In one example the bispecific antibody shows one or more properties selected from the group of

a) binding to MKN-45 cells with an EC50 value of 0.5 nM to 50 nM,

b) killing MKN-45, HPAF-II, or LS174T cells in an assay containing human PBMC in a concentration dependent manner with an EC50 value of 0.01 to 10 nM,

c) binding to CEACAM5 expressing PEAK cells, but not cross reacting with CEACAM8 expressing PEAK cells,

d) the killing EC50 in the TDCC assay (Example 8) when LS174T tumor cells are used as target cells is not increased by more than a factor 5 in presence of 1 µg/mL of sCEA,

e) inhibiting tumor growth in a HPAF-II model by 25% or more compared to the control group (vehicle only),

f) competing with an anti-CEA antibody, comprising as VL and VH domains VL and VH of sequences SEQ ID NOs: 48 and 49 (MEDI), and

g) comprising in each subunit of the Fc domain amino acid substitutions L234A, L235A and P329A (Kabat EU index numbering).

In one example the bispecific antibody shows the properties of a) to d). In one example the bispecific antibody shows all properties of a) to f). In one example the bispecific antibody shows the properties of a) to d) and g). In one example the bispecific antibody shows the properties of a) to d) and f) and g). In one example the bispecific antibody shows all properties of a) to g).

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a common heavy chain (cHC) and comprises as variable region a variable region which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises

i) a kappa light chain constant region (CL), and a light chain variable region (VL), comprising as CDRL1, CDRL2 and CDRL3, a CDRL1 of SEQ ID NO: 32, a CDRL2 of SEQ ID NO: 33 and a CDRL3 of SEQ ID NO: 34 or a light chain variable region derived from SEQ ID NO: 31 by oligonucleotide-directed mutagenesis using degenerated oligonucleotides,

c) the second binding part comprises a light chain variable region, comprising as CDRL1, CDRL2 and CDRL3 a group of CDRs selected from the group consisting of

I) CDRL1 of SEQ ID NO: 6, a CDRL2 of SEQ ID NO: 7 and a CDRL3 of SEQ ID NO: 8,

II) CDRL1 of SEQ ID NO: 10, a CDRL2 of SEQ ID NO: 11 and a CDRL3 of SEQ ID NO: 12,

III) CDRL1 of SEQ ID NO: 14, a CDRL2 of SEQ ID NO: 15 and a CDRL3 of SEQ ID NO: 16,

IV) CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20, and

V) CDRL1 of SEQ ID NO: 22, a CDRL2 of SEQ ID NO: 23 and a CDRL3 of SEQ ID NO: 24.

d) the second binding part comprises a lambda light chain constant region.

In one example the second binding part in c) comprises a light chain selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, and 29.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a heavy chain which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises a light chain variable region (VL), comprising as CDRs a CDRL1 derived from SEQ ID NO: 32 by oligonucleotide-directed mutagenesis using

degenerated oligonucleotides and comprising up to four amino acid substitutions, a CDRL2 derived from SEQ ID NO: 33 by oligonucleotide-directed mutagenesis using degenerated oligonucleotides and comprising up to four amino acid substitutions, a CDRL3 derived from SEQ ID NO: 34 by oligonucleotide-directed mutagenesis using degenerated oligonucleotides and comprising up to four amino acid substitutions,

c) the second binding part comprises a light chain variable region, comprising as CDRL1, CDRL2 and CDRL3 a CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20.

Such bispecific antibodies are, but not limited, bispecific anti CEAxCD3 antibodies AB13L3-1, AB14L3-1, AB15L3-1, AB17L3-1, AB20L3-1, AB54L3-1, AB60L3-1, AB66L3-1, AB71L3-1, AB72L3-1, and AB73L3-1.

In one aspect, the present disclosure refers to a bispecific antibody comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that:

a) the first binding part comprises a heavy chain variable region VH, which comprises as CDRH1, CDRH2, and CDRH3 a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b1) a CDRL1 of SEQ ID NO: 90, CDRL2 of SEQ ID NO: 91, and CDRL3 of SEQ ID NO: 92,

b2) a CDRL1 of SEQ ID NO: 96, CDRL2 of SEQ ID NO: 97, and CDRL3 of SEQ ID NO: 98,

b3) a CDRL1 of SEQ ID NO: 99, CDRL2 of SEQ ID NO: 100, and CDRL3 of SEQ ID NO: 101,

b4) a CDRL1 of SEQ ID NO: 102, CDRL2 of SEQ ID NO: 103, and CDRL3 of SEQ ID NO: 104,

b5) a CDRL1 of SEQ ID NO: 105, CDRL2 of SEQ ID NO: 106, and CDRL3 of SEQ ID NO: 107,
b6) a CDRL1 of SEQ ID NO: 108, CDRL2 of SEQ ID NO: 109, and CDRL3 of SEQ ID NO: 110, and
b7) a CDRL1 of SEQ ID NO: 111, CDRL2 of SEQ ID NO: 112, and CDRL3 of SEQ ID NO: 113, and
c) the second binding part comprises a heavy chain variable region VH comprising a CDRH1 of SEQ ID NO: 2, CDRH2 of SEQ ID NO: 3 and CDRH3 of SEQ ID NO: 4 and a light chain variable region VL comprising a CDRL1 of SEQ ID NO: 18, CDRL2 of SEQ ID NO: 19, and CDRL3 of SEQ ID NO: 20.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that:
a) the first binding part comprises a heavy chain variable region VH, which comprises as CDRH1, CDRH2, and CDRH3 a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,
b) the first binding part comprises as light chain variable region a light chain variable region comprising a CDRL set selected from the group consisting of
b1) a CDRL1 of SEQ ID NO: 90, CDRL2 of SEQ ID NO: 91, and CDRL3 of SEQ ID NO: 92,
b2) a CDRL1 of SEQ ID NO: 96, CDRL2 of SEQ ID NO: 97, and CDRL3 of SEQ ID NO: 98,
b3) a CDRL1 of SEQ ID NO: 99, CDRL2 of SEQ ID NO: 100, and CDRL3 of SEQ ID NO: 101,
b4) a CDRL1 of SEQ ID NO: 102, CDRL2 of SEQ ID NO: 103, and CDRL3 of SEQ ID NO: 104,
b5) a CDRL1 of SEQ ID NO: 105, CDRL2 of SEQ ID NO: 106, and CDRL3 of SEQ ID NO: 107, and
b6) a CDRL1 of SEQ ID NO: 111, CDRL2 of SEQ ID NO: 112, and CDRL3 of SEQ ID NO: 113, and
c) the second binding part comprises a heavy chain variable region VH comprising a CDRH1 of SEQ ID NO: 2, CDRH2 of SEQ ID NO: 3 and CDRH3 of SEQ ID NO: 4 and a light chain variable region VL comprising a CDRL1 of SEQ ID NO: 18, CDRL2 of SEQ ID NO: 19, and CDRL3 of SEQ ID NO: 20.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that:

a) the first binding part comprises a heavy chain variable region VH, which comprises as CDRH1, CDRH2, and CDRH3 a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises as light chain variable region a light chain variable region comprising a CDRL set selected from the group consisting of

b1) a CDRL1 of SEQ ID NO: 90, CDRL2 of SEQ ID NO: 91, and CDRL3 of SEQ ID NO: 92,

b2) a CDRL1 of SEQ ID NO: 105, CDRL2 of SEQ ID NO: 106, and CDRL3 of SEQ ID NO: 107, and

b3) a CDRL1 of SEQ ID NO: 111, CDRL2 of SEQ ID NO: 112, and CDRL3 of SEQ ID NO: 113, and

c) the second binding part comprises a heavy chain variable region VH comprising a CDRH1 of SEQ ID NO: 2, CDRH2 of SEQ ID NO: 3 and CDRH3 of SEQ ID NO: 4 and a light chain variable region VL comprising a CDRL1 of SEQ ID NO: 18, CDRL2 of SEQ ID NO: 19, and CDRL3 of SEQ ID NO: 20.

In one example, the disclosure relates to a bispecific antibody according to the disclosure, characterized in comprising

a) in the first binding part a heavy chain variable region VH having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 1, comprising a CDR1 of SEQ ID NO: 2, a CDR2 of SEQ ID NO: 3, and a CDR3 of SEQ ID NO: 4, and

b) a light chain variable region VL selected from the group consisting of

b1) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 31 and comprising a CDRL1 of SEQ ID NO: 32, CDRL2 of SEQ ID NO: 33, and CDRL3 of SEQ ID NO: 34,

b2) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 114 and comprising a CDRL1 of SEQ ID NO: 81, CDRL2 of SEQ ID NO: 82, and CDRL3 of SEQ ID NO: 83,

b3) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 115 and a CDRL1 of SEQ ID NO: 84, CDRL2 of SEQ ID NO: 85, and CDRL3 of SEQ ID NO: 86,

b4) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 116 and a CDRL1 of SEQ ID NO: 87, CDRL2 of SEQ ID NO: 88, and CDRL3 of SEQ ID NO: 89,

b5) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 117 and a CDRL1 of SEQ ID NO: 90, CDRL2 of SEQ ID NO: 91, and CDRL3 of SEQ ID NO: 92,

b6) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 118 and a CDRL1 of SEQ ID NO: 93, CDRL2 of SEQ ID NO: 94, and CDRL3 of SEQ ID NO: 95,

b7) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 119 and a CDRL1 of SEQ ID NO: 96, CDRL2 of SEQ ID NO: 97, and CDRL3 of SEQ ID NO: 98,

b8) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 120 and a CDRL1 of SEQ ID NO: 99, CDRL2 of SEQ ID NO: 100, and CDRL3 of SEQ ID NO: 101,

b9) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 121 and a CDRL1 of SEQ ID NO: 102, CDRL2 of SEQ ID NO: 103, and CDRL3 of SEQ ID NO: 104,

b10) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 122 and a CDRL1 of SEQ ID NO: 105, CDRL2 of SEQ ID NO: 106, and CDRL3 of SEQ ID NO: 107, and

b11) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 123 and a CDRL1 of SEQ ID NO: 108, CDRL2 of SEQ ID NO: 109, and CDRL3 of SEQ ID NO: 110, and

b12) a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 124 and a CDRL1 of SEQ ID NO: 111, CDRL2 of SEQ ID NO: 112, and CDRL3 of SEQ ID NO: 113,

c) the second binding part a heavy chain variable region VH having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 1, comprising a CDR1 of SEQ ID NO: 2, a CDR2 of SEQ ID

NO: 3, and a CDR3 of SEQ ID NO: 4, and a light chain variable region VL having 97%, 98%, 99%, or 100% amino acid identity to SEQ ID NO: 17, comprising a CDRL1 of SEQ ID NO: 18, CDRL2 of SEQ ID NO: 19, and CDRL3 of SEQ ID NO: 20.

In one example, the disclosure describes a bispecific antibody as disclosed herein, characterized in comprising

a) in the first binding part a heavy chain variable region VH SEQ ID NO: 1, and

b) a light chain variable region VL selected from the group consisting of

b1) a light chain variable region VL of SEQ ID NO: 31,

b2) a light chain variable region VL of SEQ ID NO: 114,

b3) a light chain variable region VL of SEQ ID NO: 115,

b4) a light chain variable region VL of SEQ ID NO: 116,

b5) a light chain variable region VL of SEQ ID NO: 117,

b6) a light chain variable region VL of SEQ ID NO: 118,

b7) a light chain variable region VL of SEQ ID NO: 119,

b8) a light chain variable region VL of SEQ ID NO: 120,

b9) a light chain variable region VL of SEQ ID NO: 121,

b10) a light chain variable region VL of SEQ ID NO: 122,

b11) a light chain variable region VL of SEQ ID NO: 123, and

b12) a light chain variable region VL of SEQ ID NO: 124, and

c) in the second binding part a heavy chain variable region VH of SEQ ID NO: 1 and a light chain variable region VL of SEQ ID NO: 17.

In one example, the disclosure relates to a bispecific antibody according to the disclosure, characterized in comprising

a) in the first binding part a heavy chain variable region VH SEQ ID NO: 1, and

b) a light chain variable region VL selected from the group consisting of

b1) a light chain variable region VL of SEQ ID NO: 117,

b2) a light chain variable region VL of SEQ ID NO: 119,

b3) a light chain variable region VL of SEQ ID NO: 120,

b4) a light chain variable region VL of SEQ ID NO: 121,

b5) a light chain variable region VL of SEQ ID NO: 122, and

b6) a light chain variable region VL of SEQ ID NO: 124, and

c) in the second binding part a heavy chain variable region VH of SEQ ID NO: 1 and a light chain variable region VL of SEQ ID NO: 17.

In one example, the disclosure refers to a bispecific antibody , characterized in comprising

a) in the first binding part a heavy chain variable region VH SEQ ID NO: 1, and

b) a light chain variable region VL selected from the group consisting of

b1) a light chain variable region VL of SEQ ID NO: 117,

b2) a light chain variable region VL of SEQ ID NO: 122, and

b3) a light chain variable region VL of SEQ ID NO: 124, and

c) in the second binding part a heavy chain variable region VH of SEQ ID NO: 1 and a light chain variable region VL of SEQ ID NO: 17.

In one example, there is disclosed a bispecific antibody comprising in the first binding part

a) a heavy chain variable region VH SEQ ID NO: 1, and

b) a light chain selected from the group consisting of

b1) the light chain of SEQ ID NO: 40

b2) the light chain of SEQ ID NO: 125,

b3) the light chain of SEQ ID NO: 126,

b4) the light chain of SEQ ID NO: 127,

b5) the light chain of SEQ ID NO: 128,

b6) the light chain of SEQ ID NO: 129,

b7) the light chain of SEQ ID NO: 130,

b8) the light chain of SEQ ID NO: 131

b9) the light chain of SEQ ID NO: 132,

b10) the light chain of SEQ ID NO: 133, and

b11) the light chain of SEQ ID NO: 134, and

b12) the light chain of SEQ ID NO: 135, and

c) in the second binding part a heavy chain variable region VH of SEQ ID NO: 1 and a light chain of SEQ ID NO: 28.

In one example, the disclosure refers to a bispecific antibody, characterized in comprising in the first binding part

a) a common heavy chain selected from the group consisting of

a1) the heavy chain of SEQ ID NO: 43,

a2) the heavy chain of SEQ ID NO: 44, or

a3) the heavy chain of SEQ ID NO: 45, and

b) a light chain selected from the group consisting of

b1) the light chain of SEQ ID NO: 40

b2) the light chain of SEQ ID NO: 125,

b3) the light chain of SEQ ID NO: 126,

b4) the light chain of SEQ ID NO: 127,

b5) the light chain of SEQ ID NO: 128,

b6) the light chain of SEQ ID NO: 129,

b7) the light chain of SEQ ID NO: 130,

b8) the light chain of SEQ ID NO: 131

b9) the light chain of SEQ ID NO: 132,

b10) the light chain of SEQ ID NO: 133

b11) the light chain of SEQ ID NO: 134, or

b12) the light chain of SEQ ID NO: 135, and

c) in the second binding part a light chain of SEQ ID NO: 28.

In one example a bispecific antibody according to the disclosure (AB17L3-1/N) comprises a common heavy chain of SEQ ID NO: 45 (/N) and in the second binding part as light chain a light chain of SEQ ID NO: 28 (1A4 LC respectively L3-1) and in the first binding part as light chain a light chain of SEQ ID NO: 128 (AB17). In one example a bispecific antibody according to the disclosure (AB71L3-1/N) comprises a common heavy chain of SEQ ID NO: 45 (/N) and in the second binding part as light chain a light chain of SEQ ID NO: 28 (L3-1) and in the first binding part as light chain a light chain of SEQ ID NO: 133 (AB71). In one example a bispecific antibody according to the disclosure (AB73L3-1/N) comprises a common heavy chain of SEQ ID NO: 45 (/N) and in the second binding part as light chain a light chain of SEQ ID NO: 28 (L3-1) and in the first binding part as light chain a light chain of SEQ ID NO: 135 (AB73).

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a common heavy chain and comprises as variable region a variable region which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises

i) a lambda light chain constant region (CL) and

ii) a light chain variable region (VL), comprising as CDRL1, CDRL2 and CDRL3, a CDRL1 of SEQ ID NO: 36, a CDRL2 of SEQ ID NO: 37 and a CDRL3 of SEQ ID NO: 38 or a light chain variable region derived from SEQ ID NO: 35 by oligonucleotide-directed mutagenesis using degenerated oligonucleotides,

c) the second binding part comprises a light chain variable region, comprising as CDRL1, CDRL2 and CDRL3 a group of CDRs selected from the group consisting of

I) CDRL1 of SEQ ID NO: 6, a CDRL2 of SEQ ID NO: 7 and a CDRL3 of SEQ ID NO: 8,

II) CDRL1 of SEQ ID NO: 10, a CDRL2 of SEQ ID NO: 11 and a CDRL3 of SEQ ID NO: 12,

III) CDRL1 of SEQ ID NO: 14, a CDRL2 of SEQ ID NO: 15 and a CDRL3 of SEQ ID NO: 16,

IV) CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20, and

V) CDRL1 of SEQ ID NO: 22, a CDRL2 of SEQ ID NO: 23 and a CDRL3 of SEQ ID NO: 24.

d) the second binding part comprises a hybrid-kappa chain constant region.

In one example the second binding part in c) comprises a light chain selected from the group consisting of SEQ ID NOs: 67, 68, 69, 70, and 71

In one example the second binding part can comprise as light chain constant region a lambda light chain constant region of SEQ ID NO: 41; in that case the first binding part comprises in one example as light chain constant region a hybrid-kappa light chain region of SEQ ID NO: 58. In one example the arm carrying the hybrid light chain constant region is based on the overall properties of the bsAb, including but not limited to stability and productivity.

In one example the light chain variable region of the first binding part is derived from SEQ ID NO: 35 by oligonucleotide-directed mutagenesis using degenerated oligonucleotides, the common heavy chain is of SEQ ID NO: 45, and the variable region of the common heavy chain is of SEQ ID NO: 1.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a common heavy chain and comprises as variable region a variable region which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises

i) a kappa light chain constant region (CL), and

ii) a light chain variable region (VL), comprising as CDRL1, CDRL2 and CDRL3, a CDRL1 of SEQ ID NO: 64, a CDRL2 of SEQ ID NO: 65 and a CDRL3 of SEQ ID NO: 66 or a light chain variable region derived from SEQ ID NO: 63 by oligonucleotide-directed mutagenesis,

c) the second binding part comprises a light chain variable region, comprising as CDRL1, CDRL2 and CDRL3 a group of CDRs selected from the group consisting of

I) CDRL1 of SEQ ID NO: 6, a CDRL2 of SEQ ID NO: 7 and a CDRL3 of SEQ ID NO: 8,

II) CDRL1 of SEQ ID NO: 10, a CDRL2 of SEQ ID NO: 11 and a CDRL3 of SEQ ID NO: 12,

III) CDRL1 of SEQ ID NO: 14, a CDRL2 of SEQ ID NO: 15 and a CDRL3 of SEQ ID NO: 16,

IV) CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20, and

V) CDRL1 of SEQ ID NO: 22, a CDRL2 of SEQ ID NO: 23 and a CDRL3 of SEQ ID NO: 24.

d) the second binding part comprises a lambda light chain constant region.

In one example the second binding part in c) comprises a light chain selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, and 29.

In one example of the disclosure the light chain variable region of the first binding part is derived from SEQ ID NO: 63 by oligonucleotide-directed mutagenesis using degenerated oligonucleotides, the common heavy chain is of SEQ ID NO: 45.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a common heavy chain and comprises as variable region a variable region which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part a lambda light chain constant region (CL), and a light chain variable region (VL), comprising as CDRL1, CDRL2 and CDRL3, a CDRL1 of SEQ ID NO: 60, a CDRL2 of SEQ ID NO: 61 and a CDRL3 of SEQ ID NO: 62 or a light chain variable region derived from SEQ ID NO: 59 by oligonucleotide-directed mutagenesis using degenerated oligonucleotides,

c) the second binding part comprises a light chain variable region, comprising as CDRL1, CDRL2 and CDRL3 a group of CDRs selected from the group consisting of

I) CDRL1 of SEQ ID NO: 6, a CDRL2 of SEQ ID NO: 7 and a CDRL3 of SEQ ID NO: 8,

II) CDRL1 of SEQ ID NO: 10, a CDRL2 of SEQ ID NO: 11 and a CDRL3 of SEQ ID NO: 12,

III) CDRL1 of SEQ ID NO: 14, a CDRL2 of SEQ ID NO: 15 and a CDRL3 of SEQ ID NO: 16,

IV) CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20, and

V) CDRL1 of SEQ ID NO: 22, a CDRL2 of SEQ ID NO: 23 and a CDRL3 of SEQ ID NO: 24.

d) the second binding part comprises a hybrid-kappa chain constant region.

In a further example of the disclosure, the second binding part comprise as light chain constant region a lambda light constant chain region of SEQ ID NO: 41; in that case the first binding part comprises in one example as light chain constant region a hybrid-kappa light chain constant region of SEQ ID NO: 58. The choice of the arm carrying the hybrid light chain constant region is based on the overall properties of the final bsAb, including but not limited to stability and productivity.

In one example the light chain variable region of the first binding part is derived from SEQ ID NO: 59 by oligonucleotide-directed mutagenesis using degenerated oligonucleotides.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a common heavy chain) and comprises as variable region a variable region which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises a light chain constant region of human kappa type and a light chain variable region of human kappa type, comprising as CDRL1, CDRL2 and CDRL3 a CDRL1 of SEQ ID NO: 32 with substitution of 0, 1, 2, 3, or 4 amino acids, a CDRL2 of SEQ ID NO: 33 with substitution of 0, 1, 2, 3 or 4 amino acids, and a CDRL3 of SEQ ID NO: 34 with substitution of 0, 1, 2, 3, 4, or 5 amino acids,

c) the second binding part comprises a light chain constant region of human lambda type and a light chain variable region of human lambda type, comprising as CDRL1, CDRL2 and CDRL3 a group of CDRs selected from the group consisting of

I) CDRL1 of SEQ ID NO: 6, a CDRL2 of SEQ ID NO: 7 and a CDRL3 of SEQ ID NO: 8,

II) CDRL1 of SEQ ID NO: 10, a CDRL2 of SEQ ID NO: 11 and a CDRL3 of SEQ ID NO: 12,

III) CDRL1 of SEQ ID NO: 14, a CDRL2 of SEQ ID NO: 15 and a CDRL3 of SEQ ID NO: 16,

IV) CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20, and

V) CDRL1 of SEQ ID NO: 22, a CDRL2 of SEQ ID NO: 23 and a CDRL3 of SEQ ID NO: 24.

In one example the second binding part in c) comprises a light chain selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, and 29.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a common heavy chain and comprises as variable region a variable region which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises light chain constant region of human lambda type and a light chain variable region of human lambda type, comprising as CDRL1, CDRL2 and CDRL3, a CDRL set selected from the group consisting of a CDRL1 of SEQ ID NO: 36 with substitution of 0, 1, 2, 3, 4, or 5 amino acids, a CDRL2 of SEQ ID NO: 37 with substitution of 0, 1, 2, 3, 4, or 5 amino acids, and a CDRL3 of SEQ ID NO: 38, with substitution of 0, 1, 2, 3, 4, or 5 amino acids,

c) the second binding part comprises a hybrid kappa light chain constant region and a light chain variable region of human lambda type, comprising as CDRL1, CDRL2 and CDRL3 a group of CDRs selected from the group consisting of

I) CDRL1 of SEQ ID NO: 6, a CDRL2 of SEQ ID NO: 7 and a CDRL3 of SEQ ID NO: 8,

II) CDRL1 of SEQ ID NO: 10, a CDRL2 of SEQ ID NO: 11 and a CDRL3 of SEQ ID NO: 12,

III) CDRL1 of SEQ ID NO: 14, a CDRL2 of SEQ ID NO: 15 and a CDRL3 of SEQ ID NO: 16,

IV) CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20, and

V) CDRL1 of SEQ ID NO: 22, a CDRL2 of SEQ ID NO: 23 and a CDRL3 of SEQ ID NO: 24.

In one example the second binding part in c) comprises a light chain selected from the group consisting of SEQ ID NOs: 67, 68, 69, 70, and 71.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a common heavy chain and comprises as variable region a variable region which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises a light chain constant region of human lambda type and a light chain variable region of human lambda type, comprising as CDRL1, CDRL2 and CDRL3, a CDRL set selected from the group consisting of a CDRL1 of SEQ ID NO: 60 with substitution of 0, 1, 2, 3, or 4 amino acids, a CDRL2 of SEQ ID NO: 61 with substitution of 0, 1, 2, 3 or 4

amino acids, and a CDRL3 of SEQ ID NO: 62 with substitution of 0, 1, 2, 3, 4, or 5 amino acids, and

c) the second binding part comprises a light chain constant region of human kappa type, and a light chain variable region of human lambda type, comprising as CDRL1, CDRL2 and CDRL3 a group of CDRs selected from the group consisting of

I) CDRL1 of SEQ ID NO: 6, a CDRL2 of SEQ ID NO: 7 and a CDRL3 of SEQ ID NO: 8,

II) CDRL1 of SEQ ID NO: 10, a CDRL2 of SEQ ID NO: 11 and a CDRL3 of SEQ ID NO: 12,

III) CDRL1 of SEQ ID NO: 14, a CDRL2 of SEQ ID NO: 15 and a CDRL3 of SEQ ID NO: 16,

IV) CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20, and

V) CDRL1 of SEQ ID NO: 22, a CDRL2 of SEQ ID NO: 23 and a CDRL3 of SEQ ID NO: 24.

In one example the second binding part in c) comprises a light chain selected from the group consisting of SEQ ID NOs: 67, 68, 69, 70, and 71.

In one example the bispecific antibody is characterized in comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3ε, characterized in that

a) the first binding part and the second binding part comprise each as heavy chain a (common heavy chain and comprises as variable region a variable region which comprises as CDRH1, CDRH2, and CDRH3, a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises a light chain constant region of human kappa type, and a light chain variable region of human kappa type, comprising as CDRL1, CDRL2 and CDRL3, a CDRL set selected from the group consisting of a CDRL1 of SEQ ID NO: 64 with substitution of 0, 1, 2, 3, 4, or 5 amino acids, a CDRL2 of SEQ ID NO: 65 with substitution of 0, 1, 2, 3, 4, or 5 amino acids, and a CDRL3 of SEQ ID NO: 66, and with substitution of 0, 1, 2, 3, 4, or 5 amino acids

c) the second binding part comprises a light chain constant region of human lambda type, and a light chain variable region of human lambda type, comprising as CDRL1, CDRL2 and CDRL3 a group of CDRs selected from the group consisting of I) CDRL1 of SEQ ID NO: 6, a CDRL2 of SEQ ID NO: 7 and a CDRL3 of SEQ ID NO: 8,

II) CDRL1 of SEQ ID NO: 10, a CDRL2 of SEQ ID NO: 11 and a CDRL3 of SEQ ID NO: 12,

III) CDRL1 of SEQ ID NO: 14, a CDRL2 of SEQ ID NO: 15 and a CDRL3 of SEQ ID NO: 16,
IV) CDRL1 of SEQ ID NO: 18, a CDRL2 of SEQ ID NO: 19 and a CDRL3 of SEQ ID NO: 20, and
V) CDRL1 of SEQ ID NO: 22, a CDRL2 of SEQ ID NO: 23 and a CDRL3 of SEQ ID NO: 24.
In one example the second binding part in c) comprises a light chain selected from the group consisting of SEQ ID NOs: 25, 26, 27, 28, and 29.

In one example, the bispecific antibody comprises a first binding part, specifically binding to human CEACAM5, and a second binding part, specifically binding to human CD3ε, wherein:
a) the first binding part comprises a heavy chain variable region (VH), which comprises a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,
b) the first binding part comprises a light chain variable region (VL) comprising a CDRL set in which the CDRL1 has the consensus sequence of SEQ ID NO: 136, the CDRL2 has the consensus sequence of SEQ ID NO: 137, and the CDRL3 has the consensus sequence of SEQ ID NO: 138, and
c) the second binding part comprises a VH comprising a CDRH1 of SEQ ID NO: 2, CDRH2 of SEQ ID NO: 3 and CDRH3 of SEQ ID NO: 4.

In one example the first binding part comprises as variable light chain framework sequence the framework sequence of SEQ ID NO: 31. In one example the first binding part comprises as variable light chain framework sequence the framework sequence of SEQ ID NO: 35. In one example the first binding part comprises as variable light chain framework sequence the framework sequence of SEQ ID NO: 59. In one example the first binding part comprises as variable light chain framework sequence the framework sequence of SEQ ID NO: 63.

In one example, the first binding part specifically binding to CEA, comprises as heavy chain variable region a heavy chain variable region of an amino acid sequence SEQ ID NO: 1 and as light chain variable region a light chain variable region of an amino acid sequence that is 98%, 99% or 100% identical to an amino acid sequence selected from the group of SEQ ID NO: 31 or SEQ ID NO: 35, SEQ ID NO: 59 or SEQ ID NO: 63, and the second binding part specifically binding to CD3, comprises as heavy chain variable region a heavy chain variable region of amino acid sequence SEQ ID NO: 1 and as light chain variable region a light chain variable region of

amino acid sequence selected from the group consisting of SEQ ID NO: 5, SEQ ID NO: 9, SEQ ID NO: 13¸ SEQ ID NO: 17¸ and SEQ ID NO: 21.

In one example the bispecific antibody according to the disclosure is characterized in comprising a first binding part specific for CEA, comprising a kappa light chain variable domain and a kappa light chain constant domain and a second binding part specific for CD3ε, comprising a lambda light chain variable domain and a lambda light chain constant domain.

In one example the bispecific antibody according to the disclosure is characterized in comprising a first binding part specific for CEA, comprising a lambda light chain variable domain and a kappa light chain constant domain and a second binding part specific for CD3ε, comprising a lambda light chain variable domain and a lambda light chain constant domain.

In one example the bispecific antibody according to the disclosure is characterized in comprising a first binding part specific for CEA, comprising a lambda light chain variable domain and a lambda light chain constant domain and a second binding part specific for CD3ε, comprising a lambda light chain variable domain and a kappa light chain constant domain.

In a particular example the Fc domain exhibits reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a native/wild type IgG1 Fc domain. In certain examples the Fc domain is engineered to have reduced binding affinity to an Fc receptor and/or reduced effector function, as compared to a non-engineered Fc domain. In one example, the Fc domain comprises one or more amino acid substitution that reduces binding to one or more Fc receptors and/or reduces effector functions. In one example such one or more substitutions are selected from the group consisting of Pro238, Asp265, Asp270, Asn297 (loss of Fc carbohydrate), Pro329, Leu234, Leu235, Gly236, Gly237, Ile253, Ser254, Lys288, Thr307, Gln311, Asn434, and His435 (Shields, R. L., et al., J. Biol. Chem. 276 (2001) 6591-6604; Lund, J., et al., FASEB J. 9 (1995) 115-119; Morgan, A., et al., Immunology 86 (1995) 319-324; EP 0 307 434). In one example the antibody is in regard to FcR binding of IgG4 subclass or of IgG1 or IgG2 subclass with a mutation in S228, L234, L235 and/or D265, and/ or contains the PVA236 mutation. In one example the mutations in the Fc domain are S228P, L234A, L235A, L235E and/or PVA236. In another example the mutations in Fc domain are

in IgG4 S228P and in IgG1 L234A and L235A. In one example, the one or more amino acid substitution in the Fc domain that reduces binding to one or more Fc receptors and/or reduces effector functions is at one or more position selected from the group of L234, L235, and P329 (Kabat EU index numbering). In particular examples, each subunit of the Fc domain comprises two amino acid substitutions that reduce binding to an Fc receptor and/or reduces effector function wherein said amino acid substitutions are L234A and L235A (Kabat EU index numbering). In particular examples, each subunit of the Fc domain comprises three amino acid substitutions that reduce binding to an Fc receptor and/or reduces effector function wherein said amino acid substitutions are L234A, L235A and P329A (Kabat EU index numbering). In one such example, the Fc domain is an IgG1Fc domain, particularly a human IgG1Fc domain (Kabat EU index numbering). In one example, the Fc domain is of IgG4 subclass and in one example of IgG4 subclass with mutation S228P.

In one example the Fc receptor is an Fcγ receptor. In one example the Fc receptor is a human Fc receptor. In one example , the Fc receptor is an activating Fc receptor. In a specific example, the Fc receptor is human FcγRIIIA, FcγRI, and/or FcγRIIIA. In one example, the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC), but not limited to only ADCC.

An example of the disclosure is a kappa CL region of SEQ ID NO: 58 for use in the second binding part as CL region in the construction of a bispecific antibody comprising a common heavy chain of SEQ ID NOs: 43, 44, or 45, and a lambda CL region of SEQ ID NO: 41 as CL region in the first binding part.

An example of the disclosure is a kappa CL region of SEQ ID NO: 58 for use in the second binding part, specifically binding to CD3 as CL region in the construction of a bispecific antibody according to the disclosure, comprising a common heavy chain of SEQ ID NOs: 43, 44, or 45, and a lambda CL region of SEQ ID NO: 41 as CL region in the first binding part, specifically binding to CEACAM5.

An example of the disclosure is a kappa CL region of SEQ ID NO: 58 for use in the second binding part, specifically binding to CD3 as CL region in the construction of a bispecific

antibody according to the disclosure, comprising a common heavy chain of SEQ ID NOs: 43, 44, or 45, a variable light chain of SEQ ID NOs: 5, 9, 13, 17, or 21, and a lambda CL region of SEQ ID NO: 41 as CL region in the first binding part, specifically binding to CEACAM5.

A further example of the disclosure is an oligonucleotide, selected from the group consisting of SEQ ID NOs: 76, 77, 78, and 79 for use in the antibody affinity maturation by oligonucleotide-directed mutagenesis using degenerated oligonucleotides of the respective light chain variable region of SEQ ID NOs: 31, 35, 59, and 63.

In another aspect the present disclosure refers to a method of producing the bispecific of the invention, comprising the steps of a) culturing the host cell of the invention under conditions suitable for the expression of the bispecific antibody and b) recovering the bispecific antibody. The disclosure also encompasses a bispecific antibody produced by the method of the disclosure.

The disclsoure further provides a pharmaceutical composition comprising the bispecific antibody of the disclosure and a pharmaceutically acceptable carrier. Also encompassed by the disclosure are methods of using the bispecific antibody and pharmaceutical composition of the disclosure. In one aspect the disclosure provides a bispecific antibody or a pharmaceutical composition of the disclosure for use as a medicament. In one aspect is provided a bispecific antibody or a pharmaceutical composition according to the disclosure for use in the treatment of a disease in an individual in need thereof. In a specific example the disease is cancer.

Also provided is a bispecific antibody of the disclosure for use in the manufacture of a medicament for the treatment of a disease in an individual in need thereof; as well as a method of treating a disease in an individual, comprising administering to said individual a therapeutically effective amount of a composition comprising the Bispecific antibody according to the disclosure in a pharmaceutically acceptable form. In a specific example the disease is cancer. In any of the above examples the individual preferably is a mammal, particularly a human.

The disclosure also describes a method for inducing lysis of a target cell, particularly a tumor cell, comprising contacting a target cell with a bispecific antibody of the disclosure in the presence of a T cell, particularly a cytotoxic T cell.

A further example of the disclosure is the bispecific antibody according to the disclosure for use in the manufacture of a medicament for treating a subject having a cancer that expresses CEA.

A further example of the disclosure is the bispecific antibody according to the disclosure for use in the manufacture of a medicament according to the disclosure, characterized in that the cancer is selected from the group consisting of colorectal cancer, non-small cell lung cancer (NSCLC), esophageal cancer, gastric/esophageal junction cancer, pancreatic cancer and breast cancer.

A further example of the disclosure is a bispecific antibody according to the disclosure for use in simultaneous, separate, or sequential combination with an anti-CD47 antibody. In one example the anti-CD47 antibody is Magrolimab, ALX148 or TTI-621 and/or TTI-622.

A further example of the disclosure is a bispecific antibody according to the disclosure, for use in simultaneous, separate, or sequential combination with a second bispecific antibody comprising a third binding part specifically binding to human CEACAM5, and a fourth binding part specifically binding to human CD47 in the treatment of a subject having a cancer that expresses CEA.

Such second bispecific CEAxCD47 antibodies are described in PCT/IB2019/054559 and US16/428,359.

A further example of the disclosure is a bispecific antibody according to the disclosure for use according to the disclosure, characterized in that the bispecific antibody according to the disclosure and the second bispecific CEAxCD47 antibody are administered to said subject alternately in 6 to 15 day intervals, but not limited to such intervals.

A further example of the disclosure is a first bispecific antibody according to the disclosure, comprising a first binding part, specifically binding to human CEACAM5 and a second binding part, specifically binding to human CD3 according to the disclosure, and a second bispecific antibody CEAxCD47 for use in the treatment of cancer according to the disclosure, characterized

in that said cancer is colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, esophageal cancer, pancreatic cancer and breast cancer.

A further example of the disclosure is a composition comprising a bispecific antibody according to the disclosure, characterized in not competing with said second CEAxCD47 bispecific antibody as defined above for use in the treatment of a subject having a cancer that expresses CEA.

A further example of the disclosure is a method for the treatment of a human patient diagnosed with a tumor (cancer), especially a solid tumor, especially a solid cancer that expresses CEA, especially colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, esophageal cancer, pancreatic cancer and breast cancer, comprising administering an effective amount of an bispecific antibody according to the disclosure and a second bispecific antibody as described in PCT/IB2019/054559 and US16/428,359, against CEA and CD47, to the human patient, the method comprising subsequently:

administering to the patient a dose of 0.1 to 30 mg/kg, in a further example of 0.5 to 10 mg/kg, in a further example of 1 to 10 mg/kg of said second anti CEAxCD47 antibody, e.g. weekly over 4 to 12 weeks,

administering to the patient said second antibody q1, q2w, q3w or optionally q4w,

administering after these 4 to 12 weeks and after additional 2 or 3 or 4 elimination half-lives of said anti CEAxCD47 antibody to the patient a dose of 0.1 to 10 mg/kg of an antibody according to the disclosure,

administering to the patient said antibody according to the disclosure q1, q2w, q3w or optionally q4w,

waiting 2 or 3 or 4 elimination half-lives of said antibody according to the disclosure and then optionally repeating said cycle of CEA x CD47 bispecific antibody administration followed by CEA x CD3 bispecific antibody administration and optionally repeat again that cycle.

This “alternating” method is applied if the antibody of the disclosure and the second bispecific antibody are competitive regarding binding to CEA.

In case said CEA x CD47 bispecific antibody and the CEA x CD3 bispecific antibody according to this disclosure are not competitive, the two bispecific antibodies can also be administered in a manner ("simultaneous manner") that the patient experiences therapeutically effective plasma and tissue concentrations of both bispecific antibodies in parallel, e.g. by administration to the patient at about the same time a dose of 0.1 to 30 mg/kg, in a further example of 0.5 to 10 mg/kg, in a further example of 1 to 10 mg/kg of the CEA x CD47 bispecific antibody and 0.1 to 10 mg/kg of the CEA x CD3 bispecific antibody of this disclosure, followed by one or more of these combined administrations at a frequency of q1w or q2w or q3w or optionally q4w.

The term "q1w" means administration once a week; q2w means administration every two weeks etc.

In a further aspect, is the present disclosure refers to a pharmaceutical composition comprising a bispecific antibody as disclosed herein and a pharmaceutically acceptable excipient or carrier.

In a further aspect the present disclosure refers to a pharmaceutical composition comprising an antibody as disclosed herein for use as a medicament.

A further preferred example of the disclosure is a pharmaceutical composition comprising an antibody according to the disclosure for use as a medicament in the treatment of solid tumor disorders expressing CEA.

A further preferred example of the disclosure is a pharmaceutical composition comprising an antibody according to the disclosure for use as a medicament in the treatment of colorectal cancer, NSCLC (non-small cell lung cancer), gastric cancer, esophageal cancer, pancreatic cancer or breast cancer.

A further example of the disclosure is a composition according to the disclosure, characterized in that the cancer is colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, esophageal cancer, pancreatic cancer, or breast cancer.

A further example of the disclosure is an antibody according to the disclosure for use in the manufacture of a pharmaceutical composition.

A further example of the disclosure is an antibody according to the disclosure and a pharmaceutically acceptable excipient or carrier for use in the manufacture of a pharmaceutical composition.

A further example of the disclosure is of an antibody according to the disclosure for use in the manufacture of a medicament in the treatment of solid tumor disorders.

A further example of the disclosure is of an antibody according to the disclosure for use in the treatment of colorectal cancer, NSCLC (non-small cell lung cancer), gastric cancer, esophageal cancer, pancreatic cancer or breast cancer.

Another example of the disclosure describesa method of inducing cell lysis of a tumor cell comprising contacting the tumor cell with the bispecific antibody of any of above described examples. In some examples, the tumor cell is a colorectal cancer cell, NSCLC (non-small cell lung cancer), gastric cancer cell, esophageal cancer cell, pancreatic cancer cell or breast cancer cell.

In one example, the cell lysis is induced by T-cell directed cellular cytotoxicity (TDCC).

Another example of the disclosure describes a method of treating a subject having a cancer that expresses CEA, the method comprising administering to the subject a therapeutically effective amount of the bispecific antibody of any of above described examples.

Another exanple of the disclosure describes a method of treating a subject having a cancer that expresses CEA, the method comprising administering to the subject a therapeutically effective amount of the bispecific antibody of any of above described examples in combination with a bispecific antibody binding to human CEA and human CD47. If the CEAxCD47 antibody and the CEAxCD3 antibody are competing they will compete for the CEA receptors on the surface of the tumor cell and the receptor occupancy and efficacy for each combination partner depends on

their binding affinity and their plasma concentrations and is therefore difficult to predict and also variable over time if the concentrations of the two drugs have a different elimination half-life respectively clearance from the body. Therefore, competing CEAxCD3 and CEAxCD47 bispecific antibodies should be given sequentially (alternating). If the CEAxCD3 and CEAxCD47 bispecific antibodies are not or only minimally competing they can be not only given sequentially but also in parallel (simultaneously) which may well be an advantage because tumor cell killing via engagement of T-cells by the CEAxCD3 bispecific antibody and at the same time via engagement of macrophages by the CEAxCD47 bispecific antibody is expected to be additive or may be even synergistic, which means efficacy is increased if both drugs are given in parallel.

Another example of the disclosure describes a method of increasing progression free survival and/or overall survival time in a subject having a cancer that expresses CEA, said method comprising administering to said subject a therapeutically effective amount of the bispecific antibody of any of above described examples. In one example, the cancer is colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, esophageal cancer, pancreatic cancer, breast cancer, head and neck carcinoma, uterine cancer, bladder cancer, or another cancer expressing CEA.

In certain examples of these methods, the bispecific antibody is administered in combination with chemotherapy or radiation therapy. In one example, the subject is a patient suffering from colorectal cancer or lung cancer or gastric cancer, esophageal cancer, or pancreatic cancer or breast cancer or another cancer expressing CEA.

In certain examples of these methods, the bispecific antibody of the disclosure is administered to a patient in doses ranging from 0.1 to 100 mg/kg of body weight per day or per week in single or divided doses, or by continuous infusion. In certain examples, the bispecific antibody of the disclosure is administered to a patient in doses ranging from 1 to 20 mg/kg.

Another example of the disclosure describesa method of treating a subject having a cancer that expresses CEA, the method comprising administering to the subject a therapeutically effective amount of the bispecific antibody of any of above described examplesin combination with a bispecific antibody against human CEA and human CD47. In certain examples of these methods, the bispecific antibody is administered in combination with a bispecific anti-CEAxCD47 antibody in simultaneous, separate, or sequential combination. In certain

examples of these methods, the bispecific anti-CEAxCD47 antibody is administered in an alternating pattern with an antibody of the disclosure, with intervals of 6 to 15 days between administrations of the antibody of the disclosure and a bispecific anti-CEAxCD47 antibody. In certain examples, the anti-CEAxCD47 antibody is administered to a patient in doses ranging from 0.1 to 100 mg/kg of body weight per day or per week in single or divided doses, or by continuous infusion.

In certain examples of these methods, the bispecific antibody is administered in combination with a PD-1 axis antagonist in simultaneous, separate, or sequential combination. In certain examples of these methods, the bispecific antibody is administered in combination with a bispecific anti-CEAxCD47 antibody and a PD-1 axis antagonist in simultaneous, separate, or sequential combination. In certain examples, the PD-1 axis antagonist is administered to a patient in doses ranging from 0.1 to 100 mg/kg of body weight per day or per week in single or divided doses, or by continuous infusion.

Another exampl of the disclosure describesa method of increasing progression free survival time and/or overall survival time in a subject having a cancer that abnormally expresses CEA, said method comprising administering to said subject a therapeutically effective amount of the bispecific antibody of any of above described examples. In one example, the cancer is colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, esophageal cancer, pancreatic cancer or breast cancer.

In certain examples of these methods, the bispecific antibody is administered in combination with chemotherapy or radiation therapy. In one example, the subject is a cancer patient with colorectal cancer or lung cancer or gastric cancer esophageal cancer, or pancreatic cancer or breast cancer or another CEA expressing cancer.

Another example of the disclosure describesa bispecific antibody according to the disclosure for use in any of the above described methods of treatment. In one example, the cancer is selected from the group consisting of colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, esophageal cancer, pancreatic cancer and breast cancer.

Another example of the disclosure describes polynucleotides that encode the bispecific antibodies disclosed herein or a domain thereof (*e.g.*, a variable light chain region and/or variable heavy chain region) that immunospecifically binds to CEACAM5 or CD3ε. In certain aspects, provided herein are polynucleotides comprising a nucleotide sequence encoding the light chain or heavy chain of an antibody described herein. The polynucleotides can comprise nucleotide sequences encoding a heavy chain comprising the VHs or heavy chain CDRs of antibodies described herein. The polynucleotides can comprise nucleotide sequences encoding a light chain comprising the VLs or light chain CDRs of antibodies described herein.

Certain examples are vectors comprising the isolated polynucleotides disclosed herein. Certain other examples are cells comprising the isolated polynucleotides or vectors encoding the bispecific antibodies disclosed herein. In some examples, the cell is selected from the group consisting of Streptomyces, yeast, CHO, YB/20, NS0, PER-C6, HEK-293T, NIH-3T3, HeLa, BHK, Hep G2, SP2/0, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10 cell, plant cell, insect cell, and human cell in tissue culture.

Certain examples are methods of making the antibodies disclosed herein. In some examples, the method of making an antibody comprises expressing the antibody using cells comprising the isolated polynucleotides or vectors encoding the bispecific antibodies disclosed herein. In some examples, the method of making an antibody comprises culturing a cell containing an isolated polynucleotide or vector encoding the bispecific antibodies disclosed herein and isolating the antibody expressed therein.

Description of the Drawings

<u>Figure 1: Epitope binning of the new CEA binders used in the kappa/lambda (KL) CEAxCD3 bispecific antibodies (details in Example 5c)</u>

For the characterization of the new CEA binding antibodies of this invention a competitive immunoassay is used. A competitive blocking profile is created against antibodies all binding to CEACAM5 (CEA) and for which the binding epitope has already been published. SM3E, MEDI (=MEDI-565), SAR, T84.66, Labetuzumab and CH1A1A are such antibodies, for details of these antibodies and the assay see Example 5c.

Fig. 2 Agilent profile of purified bispecific antibodies

In Example 6 expression, purification and analyses of the new kappa lambda bispecific CEAxCD3 antibodies is described. Purified bispecific antibodies are analyzed by electrophoresis in denaturing and reducing conditions. The Agilent 2100 Bioanalyzer is used, the figure shows a typical result obtained for the kappa lambda antibodies of this invention AB1 L3-1/D (KL CEAxCD3 bispecific antibody with a lambda CD3 LC SEQ ID NO: 28 and a kappa CEA LC SEQ ID NO: 40 and the common HC SEQ ID NO 44) and L3-1AB8 H-CK5/D (Hybrid KL CEAxCD3 bispecific antibody with a hybrid-kappa CD3 LC SEQ ID NO: 70 and a lambda CEA LC SEQ ID NO: 42 and a common HC SEQ ID NO: 44). Y4 L3-1/D is a bispecific antibody with same CD3 arm as AB1 L3-1/D and L3-1AB8 H-CK5/D but a second arm not binding to CEA, this antibody is frequently used in pharmacological assays as a control antibody.

Fig. 3 Binding to $CD3^{POS}$ Jurkat cells (and $CD3^{NEG}$ TIB-153 cells)

Concentration dependent binding of the KL bispecific antibodies of the invention AB1 L3-1/D and L3-1AB8 H-CK5/D to CD3 expressing Jurkat cells. Both KL bispecific antibodies carry the same CD3 arm. Y4L3-1/D is a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA. Right hand figure shows no binding to the CD3 negative cell line TIB-153 even at 100 nM concentration of the bispecific antibodies.

Fig. 4 Binding to CEA^{POS} MKN-45cells (and CEA^{NEG} MKN-45_$hCEA^{KO}$ cells)

Concentration dependent binding of the KL bispecific antibodies of the invention AB1 L3-1/D and L3-1AB8 H-CK5/D to CEA expressing MKN-45 cells. Both KL bispecific antibodies carry the same CD3 arm. Y4L3-1/D, a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA, does not bind to MKN-45 cells. Right hand figure shows no binding to the MKN-45 cell line after knock-out of CEACAM5 even at 100 nM concentration.

Fig. 5 Binding to CEA^{POS} LS174T cells

Concentration dependent binding of the KL bispecific antibodies of the invention AB1 L3-1/D and L3-1AB8 H-CK5/D to CEA expressing LS 174T cells. Both KL bispecific antibodies carry the same CD3 arm. Y4L3-1/D, a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA, does not bind to the LS 174T cells.

Fig. 6 Killing of CEA^{POS} MKN-45 cells (and CEA^{NEG} MKN-45_$hCEA^{KO}$ cells)

Concentration dependent T-cell retargeted killing/lysis of MKN-45 cells by the KL bispecific antibodies of the invention AB1 L3-1/D and L3-1AB8 H-CK5/D (assay described in Example 8 a). Both KL bispecific antibodies carry the same CD3 arm. Both KL bispecific antibodies show the same killing potency. This is surprising/unexpected because AB1 L3-1/D binding to MKN-45 is much weaker than the binding of L3-1AB8 H-CK5/D (see figure 4).

Y4L3-1/D, a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA, shows only very low killing/lysis potency at approximately 100 times higher concentrations.

Right hand figure: Only very low unspecific killing/lysis of CEA knocked out MKN-45 cells by all 3 bispecific antibodies.

Fig. 7 Killing of CEA^{POS} LS-174T

Concentration dependent T-cell retargeted killing/lysis of LS-174T cells by the KL bispecific antibodies of the invention AB1 L3-1/D and L3-1AB8 H-CK5/D (assay described in Example 8 a). Both KL bispecific antibodies carry the same CD3 arm. L3-1AB8 H-CK5/D shows stronger killing potency than AB1 L3-1/D. L3-1AB8 H-CK5/D binding to LS-174T cells is more potent than the binding of AB1 L3-1/D (see figure 5).

Y4L3-1/D, a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA, shows only very low killing/lysis potency at more than 100 times higher concentrations.

Fig. 8 Binding of the monoclonal anti CEA mAb 1B4 to different recombinant proteins (ELISA).

CEA ECD = Extracellular domain of CEA; CEA A3B3 is the A3 B3 domain of CEA. Recombinant Mesothelin MSLN is used as a control to measure unspecific binding (binding not specific for ECD of CEA respectively A3B3 domain of CEA).

Fig. 9 Binding of the monoclonal anti CEA mAb C11 to different recombinant proteins (ELISA).

CEA ECD = Extracellular domain of CEA; CEA A3B3 is the A3 B3 domain of CEA. Recombinant Mesothelin MSLN is used as a control to measure unspecific binding (binding not specific for ECD of CEA respectively A3B3 domain of CEA).

Fig. 10 Binding of Lead Optimization Wave 1 antibodies to $CD3^{POS}$ Jurkat cells (and $CD3^{NEG}$ TIB-153 cells)

Concentration dependent binding of the KL bispecific antibodies of the invention AB13L3-1/N,

AB14L3-1/N, AB15L3-1/N, AB17L3-1/N and AB20L3-1/N to CD3 expressing HUT-78 cells. All KL bispecific antibodies carry the same CD3 arm. AB1L3-1/N is the parental KL bispecific antibody from which the above mentioned bsAbs were derived by lead optimization. Y4L3-1/N is a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA. TCB 2014 corresponds to another CEAxCD3 T-cell bispecific antibody described in US20140242079 which was included as reference antibody. hIgG1 corresponds to a non-binding human IgG1 antibody used as isotype control. Right hand figure shows no binding to the CD3 negative cell line JKTβ-del even at 100 nM concentration of the bispecific antibodies. Method is described in Example 7b.

Fig. 11 Binding of Lead Optimization Wave 1 antibodies to CEA^{POS} MKN-45 cells, HPAF-II cells and LS174T (ATCC® CL-188™) cells (and CEA^{NEG} MKN-45_$hCEA^{KO}$ cells)
Concentration dependent binding of the KL bispecific antibodies of the invention AB13L3-1/N, AB14L3-1/N, AB15L3-1/N, AB17L3-1/N and AB20L3-1/N to CEA positive MKN-45 (A); HPAF-II (C) and LS174T (D) or CEA negative MKN-45_$hCEA^{KO}$ (B) cells. All KL bispecific antibodies carry the same CD3 arm. AB1L3-1/N is the parental KL bispecific antibody from which the above mentioned bsAbs were derived by affinity maturation. Y4L3-1/N is a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA. TCB 2014 corresponds to another CEAxCD3 T-cell bispecific antibody described in US20140242079 which was included as reference antibody. hIgG1 corresponds to a non-binding human IgG1 antibody used as isotype control. Figure 10B show no binding to the CEA negative cell line MKN-45_$hCEA^{KO}$ cells even at 100 nM concentration of the bispecific antibodies. Binding of the antibodies of this invention to MKN-45 and/or HPAF-II cells at 100 nM concentration is 40% or more higher than binding of the TCB2014. Method described in Example 7a.

Fig. 12 Killing of CEA^{POS} MKN-45, HPAF-II, and LS174T cells (and CEA^{NEG} MKN-45_$hCEA^{KO}$ cells) by Lead Optimization Wave 1 antibodies
Concentration dependent T-cell retargeted killing/lysis of CEA positive MKN-45 (A); HPAF-II (C) and LS174T (D) or CEA negative MKN-45_$hCEA^{KO}$ (B) cells by the KL bispecific antibodies of the invention AB13L3-1/N, AB14L3-1/N, AB15L3-1/N, AB17L3-1/N and AB20L3-1/N (assay described in Example 8a). All KL bispecific antibodies carry the same CD3 arm. All KL bispecific antibodies show an improved killing as compared to the parental AB1L3-1/N antibody, from which they were derived by lead optimization, when CEA-positive cells were

used (A, C, D), but only very low unspecific killing/lysis of CEA knocked out MKN-45 cells (B). Y4L3-1/N, a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA, shows only very low killing/lysis potency at the highest concentrations tested. TCB 2014 corresponds to another CEAxCD3 T-cell bispecific antibody described in US20140242079 which was included as reference antibody. EC50 of the bispecific antibodies of this invention are lower than EC50 of TCB2014, demonstrating improved potency for tumor cell killing. Method described in Example 8a.

Fig. 13 Binding of Lead Optimization Wave 2 antibodies to $CD3^{POS}$ primary T-cells (CD4+ and CD8+) and $CD3^{NEG}$ B-cells and monocytes

Concentration dependent binding of the KL bispecific antibodies of the invention AB54L3-1/N, AB60L3-1/N, AB66L3-1/N, AB71L3-1/N, AB72L3-1/N and AB73L3-1/N to CD3 positive primary CD4+ T-cells (A) and primary CD8+ T-cells (B) or to CD3 negative B-cells (C) and Monocytes (D). All KL bispecific antibodies carry the same CD3 arm and bind similarly to the CD3-positive T-cell populations. Y4L3-1/N, a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA, binds equally well to CD3 positive T-cells. TCB 2014 corresponds to another CEAxCD3 T-cell bispecific antibody described in US20140242079 which was included as reference antibody. The anti-human CD47 antibody B6H12 (aCD47 mAb) was used as positive control (T-cells, B-cells, and monocytes express CD47). Secondary Ab only corresponds to a condition where only the detection antibody was added to the cells and serves to determine the background signal (negative control). All tested antibodies show no binding to the CD3 negative cell populations even at 200 nM concentration.

Fig. 14 Binding of Lead Optimization Wave 2 antibodies to CEA^{POS} MKN-45 cells, HPAF-II cells and LS174T cells (and CEA^{NEG} MKN-45_$hCEA^{KO}$ cells)

Concentration dependent binding of the KL bispecific antibodies of the invention AB54L3-1/N, AB60L3-1/N, AB66L3-1/N, AB71L3-1/N, AB72L3-1/N and AB73L3-1/N to CEA positive MKN-45 (A); HPAF-II (C) and LS174T (D) or CEA negative MKN-45_$hCEA^{KO}$ (B) cells. All KL bispecific antibodies carry the same CD3 arm. Y4L3-1/N is a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA. TCB 2014 corresponds to another CEAxCD3 T-cell bispecific antibody described in US20140242079 which was included as reference antibody. Figure 14B show no binding to the CEA negative cell line MKN-45_$hCEA^{KO}$ cells even at 200 nM concentration of the bispecific antibodies. For figure 14A, EC50 values are

reported in table 4. Binding of bispecific antibodies of the invention at 200 nM concentration are 40% or more higher than binding of TCB2014.

Fig. 15 Killing of CEA^{POS} MKN-45, HPAF-II and LS174T cells (and CEA^{NEG} MKN-45_$hCEA^{KO}$ cells) by Lead Optimization Wave 2 antibodies

Concentration dependent T-cell retargeted killing/lysis of CEA positive MKN-45 (A); HPAF-II (C) and LS174T (D) or CEA negative MKN-45_$hCEA^{KO}$ (B) cells by the KL bispecific antibodies of the invention AB54L3-1/N, AB60L3-1/N, AB66L3-1/N, AB71L3-1/N, AB72L3-1/N and AB73L3-1/N (assay described in Example 8a). All KL bispecific antibodies carry the same CD3 arm. Y4L3-1/N, a KL bispecific antibody with the same CD3 arm but with the second arm not binding to CEA, shows only very low killing/lysis potency at the highest tested concentrations. TCB 2014 corresponds to another CEAxCD3 T-cell bispecific antibody described in US20140242079 which was included as reference antibody. Compared to this reference antibody TCB2014, the KL bispecific antibodies of the invention AB54L3-1/N, AB60L3-1/N, AB66L3-1/N, AB71L3-1/N, AB72L3-1/N and AB73L3-1/N all show lower EC50 values, respective more potent killing of CEA positive target cells (A, C, D), but equivalently low unspecific killing/lysis of CEA knocked out MKN-45 cells (B). For figure 15A, C and D, EC50 values are reported in table 5.

Fig. 16 Secretion of Cytokine after T cell mediated killing of MKN45 tumor cells

Secretion of Perforin (B); Granzyme B (C); IFN-γ (D); TNF-α (E); IL-2 (F); IL-6 (G); IL-10 (H) mediated by the KL bispecific antibodies of the invention AB17L3-1/N, AB72L3-1/N and AB73L3-1/N after T cell mediated killing of MKN45 tumor cells (E:T (human PBMC: tumor cells) = 10:1, 48 h incubation, specific lysis is shown in (A). Method described in Example 8e.

Fig. 17 T-cell activation after T cell-mediated killing of MKN-45 tumor cells

Human CD4+ and CD8+ T-cell upregulation of CD25 (A and B) and CD69 (C and D) mediated by the KL bispecific antibodies of the invention AB17L3-1/N, AB72L3-1/N and AB73L3-1/N 2 days after T cell-mediated killing of CEA-positive MKN-45 tumor cells (killing/lysis of tumor cells shown in Fig. 16A).

Despite statistically not different tumor cell lysis at 100 nM, less T-cell activation of the antibodies of the invention compared to TCB2014 suggesting lower side effects at same tumor lysis, Method described in Example 8c.

Fig. 18 In vivo anti-tumor efficacy in the HPAF-II model in NOG mice with huPBMC transfer

In vivo anti-tumor efficacy of the KL bispecific antibodies of the invention AB17L3-1/N, AB72L3-1/N and AB73L3-1/N and of TCB2014 (all single 10 mg/kg injection) in the HPAF-II tumor cell line model in NOG mice with huPBMC transfer. Mice were randomized at day 11, when the average tumor volume was close to 150 mm^3. Results show average and SEM from 8 mice of tumor volume measured by caliper in the different study groups. No statistical difference between AB73L3-1/N and TCB2014 was found. Method described in Example 9a.

Definitions

Terms are used herein as generally used in the art, unless otherwise defined as follows.

As used herein, the term "antigen binding part, binding part" refers in its broadest sense to a part of an antibody that specifically binds an antigenic determinant such as CEA, CD47 and CD3.

More specifically, as used herein, a binding part that binds membrane-bound human carcinoembryonic antigen (CEA, same as CEACAM5) or to CD3 specifically binds to CEA or CD3, more particularly to cell surface or membrane-bound CEA or CD3. By "specifically binding, specific for, binding to" is meant that the binding is selective for the antigen and can be discriminated from unwanted or nonspecific interactions. In some embodiments, the extent of binding of an anti-target antibody to an unrelated, non-target protein is about 10-fold preferably >100-fold less than the binding of the antibody to said target as measured, e.g., by surface plasmon resonance (SPR) e.g. Biacore®, enzyme-linked immunosorbent (ELISA) or flow cytometry (FACS). Targets are the proteins discussed herein – e.g. CEA, CD47, and CD3ε.

“Specifically binding to CEA, CD3, binding to CEA, CD3” refers in one embodiment to an antibody that is capable of binding to the targets CEA resp. CD3 with sufficient affinity such that the antibody is useful as a therapeutic agent in retargeting T-cells to tumor cells via the binding of CD3, resp. CEA.

Preferably the bispecific antibody according to the invention binds to an epitope of CD3 that is conserved from different species, preferably among human and cynomolgus.

As used herein, the term "antibody" refers to an antibody comprising two heavy chains and two light chains. In one embodiment the antibody is a full length antibody. As used herein, the term “antibody heavy chain” refers to an antibody heavy chain, consisting of a variable region (variable domain) and a constant region (constant domain) as defined for a full length antibody. As used herein, the term “antibody light chain” refers to an antibody light chain, consisting of a variable region and a constant region as defined for a full length antibody. Constant light chains, useful for the present invention, are comprised in light chains as disclosed in the present invention.

The term "full length antibody" denotes an antibody consisting of two "full length antibody heavy chains" and two "full length antibody light chains". A "full length antibody heavy chain" is a polypeptide consisting in N-terminal to C- terminal direction of an antibody heavy chain variable domain (VH), an antibody constant heavy chain domain 1 (CH1), an antibody hinge region (HR), an antibody heavy chain constant domain 2 (CH2), and an antibody heavy chain constant domain 3 (CH3), abbreviated as VH-CH1-HR-CH2-CH3. A "full length antibody light chain" is a polypeptide consisting in N-terminal to C- terminal direction of an antibody light chain variable domain (VL), and an antibody light chain constant domain (CL), abbreviated as VL-CL. The antibody light chain constant domain (CL) can be κ (kappa) or λ (lambda). The two full length antibody domains are linked together via inter-polypeptide disulfide bonds between the CL domain and the CH1 domain and between the hinge regions of the full length antibody heavy chains. Examples of typical full length antibodies are natural antibodies like IgG (e.g. IgG 1 and IgG2), IgM, IgA, IgD, and IgE. The full length antibody according to the invention is in one embodiment of human IgG1 type, in one further embodiment comprising one or more amino acid substitutions in the Fc part as defined below. The full length antibody according to the invention comprise two binding parts each formed by a pair of VH and VL, one binding to CEA and the other binding to CD3.

As used herein, the term "Fc region; Fc domain" refers to a C-terminal region of an IgG heavy chain; in case of an IgG1 antibody, the C-terminal region comprises –CH2-CH3 (see above). Although the boundaries of the Fc region of an IgG heavy chain might vary slightly, the human

IgG heavy chain Fc region is usually defined to stretch from the amino acid residue at position Cys226 to the carboxyl-terminus.

Constant regions are well known in the state of the art and e.g. described by Kabat, E.A., (see e.g. Johnson, G., and Wu, T.T., Nucleic Acids Res. 28 (2000) 214-218; Kabat, E.A., et al, Proc. Natl. Acad. Sci. USA 72 (1975) 2785- 2788).

The term "epitope" includes any polypeptide determinant capable of specific binding to an antibody. In certain embodiments, epitope determinants include chemically active surface groupings of molecules such as amino acids, sugar side chains, phosphoryl, or sulfonyl, and, in certain embodiments, may have specific three dimensional structural characteristics, and or specific charge characteristics. An epitope is a region of a target that is bound by an antibody.

As used herein, the term “a common heavy chain (cHC)” refers to a polypeptide consisting in N-terminal to C-terminal direction of an antibody heavy chain variable domain (VH), an antibody heavy chain constant domain 1 (CH1), an antibody hinge region (HR), an antibody heavy chain constant domain 2 (CH2), and an antibody heavy chain constant domain 3 (CH3), abbreviated as VH-CH1-HR-CH2-CH3. Common heavy chains suitable for the bispecific antibodies according to the invention are heavy chains as described in WO2012023053, WO2013088259, WO2014087248, WO2019175658, and WO2016156537 (the entire contents of each of which are herein incorporated by reference). In one embodiment the cHC of the bispecific antibody according to the invention comprises as heavy chain CDRs a CDRL1 of SEQ ID NO: 2, a CDRL2 of SEQ ID NO: 3, and a CDRL3 of SEQ ID NO: 4. In one embodiment the cHC of the bispecific antibody according to the invention comprises as heavy chain variable region a VH region of SEQ ID NO: 1. In one embodiment the cHC of the bispecific antibody according to the invention is of SEQ ID NOs: 43, 44, or 45.

The format of the bispecific antibodies according to the invention and comprising a common heavy chain, allows the affinity purification of bispecific antibodies which are undistinguishable from a standard IgG molecule and with characteristics that are undistinguishable from a standard monoclonal antibody (see e.g. WO2013088259, WO2012023053), promising no or low immunogenicity potential in patients.

.

As used herein "AB1L3-1, AB17L3-1, AB54L3-1, AB60L3-1, AB66L3-1, AB71L3-1, AB72L3-1, AB73L3-1 and the like" refer to bispecific CEAxCD3 antibodies according to the invention, comprising a common heavy chain comprising as heavy chain CDRs the CDRs of SEQ ID NOs: 2, 3, and 4 and in the second binding part a light chain comprising as light chain CDRs the CDRs of SEQ ID NOs: 18, 19, and 20. AB1 etc. denotes therefore for a first binding part (anti-CEACAM5 binding part) and L3-1 denotes for a second binding part (anti-CD3 binding part).

In one embodiment AB1L3-1, AB17L3-1, AB54L3-1, AB60L3-1, AB66L3-1, AB71L3-1, AB72L3-1, AB73L3-1 and the like comprise a common heavy chain of SEQ ID NO: 43 (WT hIgG1) and in the second binding part as light chain a light chain of SEQ ID NO: 28. Such bispecific antibodies of the invention are designated in the examples also as AB1L3-1, AB17L3-1, AB71L3-1, AB72L3-1, AB73L3-1.

In one embodiment AB1L3-1, AB17L3-1, AB54L3-1, AB60L3-1, AB66L3-1, AB71L3-1, AB72L3-1, AB73L3-1 and the like comprise a common heavy chain of SEQ ID NO: 44 (hIgG1 with L234A + L235A mutations) and in the second binding part as light chain a light chain of SEQ ID NO: 28 Such bispecific antibodies of the invention are designated in the examples as AB1L3-1/D, AB17L3-1/D, AB71L3-1/D, AB72L3-1/D, AB73L3-1/D and the like.

In one embodiment AB1L3-1, AB17L3-1, AB54L3-1, AB60L3-1, AB66L3-1, AB71L3-1, AB72L3-1, AB73L3-1 and the like comprise a common heavy chain of SEQ ID NO: 45 (IgG1 with L234A + L235A + P329A mutations) and in the second binding part as light chain a light chain of SEQ ID NO: 28 Such bispecific antibodies of the invention are designated in the examples as AB1L3-1/N, AB17L3-1/N, AB54L3-1/N, AB60L3-1/N, AB66L3-1/N, AB71L3-1/N, AB72L3-1/N, AB73L3-1/N and the like.

Bispecific antibodies of the invention, comprising a common heavy chain, can be made for example according to WO2012023053. The methods described in WO2012023053 generate bispecific antibodies that are identical in structure to a human immunoglobulin. This type of molecule is composed of two copies of a unique heavy chain polypeptide, a first light chain

variable region fused to a Kappa constant domain and second light chain variable region fused to a Lambda constant domain.

In the bispecific antibodies of the invention one binding site displays specificity to CEA and the other site displays specificity to CD3, wherein to each the heavy and the respective light chain contribute. The light chain variable regions can be of the Lambda or Kappa family and are preferably fused to a Lambda and Kappa constant domains, respectively. This is preferred in order to avoid the generation of non-natural polypeptide junctions. However, it is also possible to obtain an antibody arm usable for the generation of bispecific antibodies of the invention by fusing a Kappa light chain variable domain to a Lambda constant domain for any of the two specificities or by fusing a Lambda light chain variable domain to a Kappa constant domain, also for any of the two specificities. The bispecific antibodies described in WO 2012023053 are "κλ Bodies". This κλ-Body format allows the affinity purification of a bispecific antibody that is undistinguishable from a standard IgG molecule with characteristics that are undistinguishable from a standard monoclonal antibody and, therefore, favorable as compared to previous formats including e.g. amino acid bridges or other unnatural elements.

An essential step of the method is the identification of two antibody Fv regions (each composed by a variable light domain and variable heavy domain) having different antigen specificities that share the same heavy chain variable domain. Numerous methods have been described for the generation of monoclonal antibodies and fragments thereof. (see, e.g., Antibodies: A Laboratory Manual, Harlow E, and Lane D, 1988, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY). Fully human antibodies are antibody molecules in which the sequence of both the light chain and the heavy chain, including the CDRs 1 and 2, arise from human genes. The CDR3 region can be of human origin or designed by synthetic means. Such antibodies are termed "human antibodies", or "fully human antibodies". Human monoclonal antibodies can be prepared by using the trioma technique; the human B-cell hybridoma technique (see Kozbor, et al., 1983 Immunol Today 4: 72); and the EBV hybridoma technique to produce human monoclonal antibodies (see Cole, et al., 1985 In: Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies may be utilized and may be produced by using human hybridomas (see Cote, et al., 1983. Proc Natl Acad Sci USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus in vitro (see Cole, et al., supra).

The term “CD3ε or CD3” as used herein relates to human CD3ε described under UniProt P07766 (CD3E_HUMAN). The term “antibody against CD3, anti CD3 antibody” relates to an antibody binding to CD3ε.

As used herein, the term "CEA, CEACAM5” refers to human carcinoembryonic antigen (CEA, CEACAM-5 or CD66e; UniProtKB - P06731) which is a cell surface glycoprotein and a tumor-associated antigen (Gold and Freedman, J Exp. Med., 121:439-462, 1965; Berinstein NL, J Clin Oncol., 20:2197-2207, 2002). As used herein, the term "CEACAM6” refers to human CEACAM6 (CD66c; UniProtKB - P40199), which is also a member of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family. As used herein, the term "CEACAM1” refers to human CEACAM1 (UniProtKB - P13688 (CEAM1_HUMAN) which is also a member of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family. As used herein, the term "CEACAM8” refers to human CEACAM8 (UniProtKB - P31997 (CEAM8_HUMAN) which is also a member of the carcinoembryonic antigen-related cell adhesion molecule (CEACAM) family. Further information and information on other members of the CEA family can be found under http://www.uniprot.org.

As used herein, the terms “specifically binding to CEA, binding to CEA, CEA binding part” refer in the context of the bispecific antibodies according to the invention to specificity for CEACAM5 on the surface of a cell. Binding to CEA on cells can be measured with gastric adenocarcinoma MKN-45 cells comprising 100.000 to 400.000 CEA copies per cell. The concentration of the antibody according to the invention is varied in an appropriate range in regard to a resulting EC50 value for binding to MKN-45 cells as defined above. The bispecific antibodies according to the invention are specifically binding to such cell membrane-bound CEACAM5.

As used herein, the term "membrane-bound human CEA" refers to human carcinoembryonic antigen (CEA) that is bound to a membrane-portion of a cell or to the surface of a cell, in particular, the surface of a tumor cell. The term "membrane-bound human CEA" may, in certain circumstances, refer to CEA which is not bound to the membrane of a cell, but which has been constructed so as to preserve the membrane bound CEA epitope to which the antibody according to the invention binds.

As used herein, the terms “no cross reactivity against CEACAM8” refer in the context of the bispecific antibodies according to the invention that the binding of the bispecific antibody according to the invention is tested on PEAK cells expressing CEACAM8 in comparison to binding to WT PEAK cells (for details see Example 1 and 5) and no crossreactivity means that the MFI measured for PEAK cells expressing CEACAM8 is no more than two times the MFI measured for WT PEAK cells. As used herein, the terms “no cross reactivity against a certain CEACAM” refer in the context of the bispecific antibodies according to the invention to said crossreactivity under the same experimental procedure and definition as described for CEACAM8.

As used herein, the term "bispecific antibody binding to human CEA and human CD3, CEAxCD3 bsAb” means a bispecific antibody binding to human CEACAM5 and CD3ε.

As used herein the term "complementarity determining region" ("CDR") describes the non-contiguous antigen combining sites (also known as antigen binding regions) found within the variable region of both heavy and light chain polypeptides. CDRs are also referred to as "hypervariable regions" and that term is used interchangeably herein with the term "CDR" in reference to the portions of the variable region that form the antigen binding regions. This particular region has been described by Kabat et al., U.S. Dept. of Health and Human Services, "Sequences of Proteins of Immunological Interest" (1983) and by Chothia et al., J. Mol. Biol. 196:901-917 (1987). Kabat et al. also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of "Kabat numbering" to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, "Kabat numbering" refers to the numbering system set forth by Kabat et al., U.S. Dept. of Health and Human Services, "Sequence of Proteins of Immunological Interest" (1983). Unless otherwise specified, references to the numbering of specific amino acid residue positions in bispecific antibody according to the invention (e.g. CDR sequences), are according to the Kabat numbering system.

As used herein the term “oligonucleotide-directed mutagenesis” relates to such method using degenerated oligonucleotides. For mutagenesis of each CDR a combination of various

degenerated oligonucleotides is used. These comprise (but are not limited to) the degenerated codons NNS, HMT, DMT, NHT.

As used herein the term “expression vector” refers to one or more vectors which comprise the heavy and light chains of the antibody according to the invention in an appropriate manner as known from the state of the art. As used herein, the term "host cell" covers any kind of cellular system which can be engineered to generate the bispecific antibodies of the present invention. In one embodiment, the host cell is engineered to allow the production of an antigen binding molecule.

As used herein the term “substitution of amino acid” refers to a substitution of one amino acid by another amino acid out of the group of the 20 proteinogenic standard amino acids.

Therapeutic Applications and Methods of Using Anti-CEAxCD3 Antibodies According to the Invention

The CEACAM x CD3 bispecific antibodies according to the invention are optimized for treatment of solid tumors, either in monotherapy or in combination therapy especially together with an anti CD47 antibody, an anti-CEAxCD47 antibody and/or PD-1 axis antagonist. The antibody according to the invention and the CD47 antibody or the CEAxCD47 antibody can be administered as described below.

In a particular embodiment, the disease resp. solid tumor is a cancer that expresses or even overexpresses CEA, including but not limited to the group of colorectal tumor, non-small cell lung tumor, gastric tumor, esophageal cancer, pancreatic tumor and breast tumor. In a particular embodiment, the tumor is a colorectal tumor. All therapeutic applications methods of use, uses, combinations, etc. described herein are especially embodiments for the treatment of these tumors/diseases.

The inventors recognize that the antibodies according to the invention show low or no ADA formation potential respectively loss of exposure due to neutralizing ADA respectively loss of efficacy.

In one embodiment, the invention provides a method of treating carcinomas (cancer, tumors, for example, human carcinomas), especially CEA expressing tumors, in vivo. This method comprises administering to a subject a pharmaceutically effective amount of a composition containing a bispecific antibody of the invention. By "subject" is meant a human subject, in one embodiment a patient suffering from cancer/tumor/carcinoma.

CEA expression in various tumor entities is generally very high, especially in colorectal carcinoma, esophageal cancer, pancreatic adenocarcinoma, gastric cancer, non-small cell lung cancer, breast cancer, head and neck carcinoma, uterine and bladder cancers among others. In healthy, normal glandular epithelia in the gastrointestinal tract, CEA is mainly expressed in a polarized pattern on the apical surface of the cells. This polarized expression pattern limits the accessibility by anti-CEA mono or bispecific antibodies which are administered systemically and therefore potential toxicity. This polarized expression pattern gets lost in the cells of gastrointestinal malignant tumors. CEA is expressed equally over the whole cell surface of the cancer cells that means cancer cells are much better accessible to an antibody of the invention than normal, healthy cells and can be selectively killed by the CEAxCD3 bispecific antibodies of the invention respectively by the combinations mentioned above.

In one embodiment the bispecific antibodies of this invention can be used in monotherapy for the treatment of advanced solid tumors, in one embodiment CEA expressing tumors. In one embodiment a bispecific antibody according to the invention is used in combination with a CEAxCD47 bsAb in simultaneous, separate, or sequential combination. In one embodiment a bispecific antibody according to the invention is used in combination with a CEAxCD47 bsAb and/or a PD-1 axis antagonist in simultaneous, separate, or sequential combination. In one embodiment a bispecific antibody according to the invention is used in combination with a PD-1 axis antagonist in simultaneous, separate, or sequential combination. Such PD-1 axis antagonists are described e.g. in WO2017118675. Such combinations attack the solid cancer by macrophages and T-cells. CD47 antibodies are e.g. described in WO2009091601, WO2009091547, WO2011143624, WO2009131453, WO2013119714, WO2015105995, WO2017181033, WO2018026600, WO2019157432, and WO2013032948 and bispecific antibody against CEA and CD47 are described in PCT/IB2019/054559 and US16/428,539.

As used herein the terms “combination, simultaneous, separate, or sequential combination” of a an antibody according to the invention and a second antibody, binding to human CD47 or to human CEA and human CD47 refer to any administration of the two antibodies (or three antibodies in case of the combination of an antibody of the invention, a CD47 mAb or a CEAxCD47 bsAb and a PD-1 axis antagonist), either separately or together, where the two or three antibodies are administered as part of an appropriate dose regimen designed to obtain the benefit of the combination therapy, for example in separate, sequential, simultaneous, concurrent, chronologically staggered or alternating administration. Thus, the two or three antibodies can be administered either as part of the same pharmaceutical composition or in separate pharmaceutical compositions. The antibody according to the invention can be administered prior to, at the same time as, or subsequent to the administration of the second bispecific antibody, or in some combination thereof. Where the antibody according to the invention is administered to the patient at repeated intervals, e.g., during a standard course of treatment, the second bispecific antibody can be administered prior to, at the same time as, or subsequent to, each administration of the antibody of the invention or some combination thereof, or at different intervals in relation to the treatment with the antibody of the invention, or in a single dose prior to, at any time during, or subsequent to the course of treatment with the antibody of the invention. In one embodiment the antibody according to the invention and the second bispecific antibody are administered in alternating administration, in one embodiment in intervals of 6 to 15 days between administration of the antibody of the invention and the second antibody. In such alternating administration the first dose can be the antibody of the invention or the second antibody.

The term "PD-1 axis antagonist" refers to an anti-PD-1 antibody or an anti-PD-Ll antibody. Anti-PD-1 antibodies are e.g. pembrolizumab (Keytruda®, MK-3475), nivolumab, pidilizumab, lambrolizumab, MEDI-0680, PDR001, and REGN2810. Anti-PD-1 antibodies are described e.g. in WO200815671, WO2013173223, WO2015026634, US7521051, US8008449, US8354509, WO20091/14335, WO2015026634, WO2008156712, WO2015026634, WO2003099196, WO2009101611, WO2010/027423, WO2010/027827, WO2010/027828, WO2008/156712, and WO2008/156712. Anti-PD-Ll antibodies are e.g. atezolizumab, MDX-1 105, durvalumab and avelumab. Anti-PD-Ll antibodies are e.g. described in WO2015026634, WO2013/019906, W02010077634, US8383796, WO2010077634, WO2007005874, and WO2016007235.

With regard to combined administration of the antibody according to the invention and the second bispecific antibody, both compounds may be present in one single dosage form or in separate dosage forms, for example in two different or identical dosage forms.

If the antibody of the invention and the second antibody are not competing in regard to CEACAM5, in one embodiment both antibodies are administered simultaneously. If the antibody of the invention and the second antibody are competing in regard to CEACAM5, in one embodiment the antibodies are administered in alternating administration.

The antibody of the invention will typically be administered to the patient in a dose regimen that provides for the most effective treatment of the cancer (from both efficacy and safety perspectives) for which the patient is being treated, as known in the art. Preferably tumor cells are attacked at the same time by T-cells and macrophages, to achieve full therapeutic potential of this approach, CEAxCD3 and CEAxCD47 bispecific antibodies should be non-competitive regarding binding to CEA on cell surface.

As discussed above, the amount of the antibody administered and the timing of the administration of the antibody of the invention can depend on the type (e.g. gender, age, weight) and condition of the patient being treated, the severity of the disease or condition being treated, and on the route of administration. For example, the antibody of the invention and the second antibody can be administered to a patient in doses ranging from 0.1 to 100 mg/kg of body weight per day or per week in single or divided doses, or by continuous infusion. In one embodiment each of the antibodies of the invention and the second antibody is administered to a patient in doses ranging from 1 to 20 mg/kg. In some instances, dosage levels below the lower limit of the aforesaid range may be adequate, while in other cases still larger doses may be employed without causing any harmful side effect.

As used herein, the term “half-life of the antibody” refers to the half-life of said antibody as measured in a usual pharmacokinetic assay. An antibody according to the invention and the second bispecific antibody against CEA and CD47 have elimination half-life of 3-14 days.

In another aspect, the invention is also directed to use of the bispecific antibody according to the invention in the treatment of disease, particularly cell proliferation disorders wherein CEA is expressed, particularly wherein CEA is abnormally expressed (e.g., overexpressed or expressed in a different pattern on the cell surface) compared to normal tissue of the same cell type. Such disorders include, but are not limited to colorectal cancer, NSCLC (non-small cell lung cancer), gastric cancer, esophageal cancer, pancreatic cancer and breast cancer. CEA expression levels may be determined by methods known in the art (e.g., via immunohistochemistry assay, immunofluorescence assay, immunoenzyme assay, ELISA, flow cytometry, radioimmunoassay etc.).

In one aspect, bispecific antibodies of the present invention can be used for targeting cells in vivo or in vitro that express CEA. The bispecific antibodies of the invention are particularly useful in the prevention of tumor formation, eradication of tumors and inhibition of tumor growth or metastasis via the induction of TDCC of tumor cells. The bispecific antibodies of the invention can be used to treat any tumor expressing CEA. Particular malignancies that can be treated with the bispecific antibodies of the invention include, but are not limited to, colorectal cancer, non-small cell lung cancer, gastric cancer, esophageal cancer, pancreatic cancer and breast cancer.

The bispecific antibodies of the invention are administered to a mammal, preferably a human, in a pharmaceutically acceptable dosage form such as those discussed below, including those that may be administered to a human intravenously as a bolus or by continuous infusion over a period of time, by intramuscular, intraperitoneal, intra-cerebrospinal, subcutaneous, intra-articular, intrasynovial, intrathecal, oral, topical, or inhalation routes. The bispecific antibodies of the invention also are suitably administered by intra tumoral, peritumoral, intralesional, or perilesional routes, to exert local as well as systemic therapeutic effects.

For the treatment of disease, the appropriate dosage of bispecific antibodies of the invention will depend on the type of disease to be treated, the severity and course of the disease, previous therapy, the patient's clinical history and response to the antibody, and the discretion of the attending physician. The bispecific antibody of the invention is suitably administered to the patient at one time or over a series of treatments. The present invention provides a method for selectively killing tumor cells expressing CEA.

This method comprises interaction of the bispecific antibodies of the invention with said tumor cells. These tumor cells may be from a human carcinoma including colorectal carcinoma, non-small cell lung carcinoma (NSCLC), gastric carcinoma, esophageal cancer, pancreatic carcinoma and breast carcinoma.

In another aspect, the invention is directed to a bispecific antibodies of the invention for use in the manufacture of a medicament for treating a disease related to abnormal CEA expression. In a particular embodiment, the disease is a cancer that expresses or even overexpresses CEA, including but not limited to colorectal tumor, non-small cell lung tumor, gastric tumor, esophageal cancer, pancreatic tumor and breast tumor. In a particular embodiment, the tumor is a colorectal tumor.

Compositions, Formulations, Dosages, and Routes of Administration

In one aspect, the present invention is directed to pharmaceutical compositions comprising the bispecific antibodies of the present invention and a pharmaceutically acceptable carrier. The present invention is further directed to such pharmaceutical compositions for use in the method of treatment of disease, such as cancer, or in the manufacture of a medicament for the treatment of disease, such as cancer. Specifically, the present invention is directed to a method for the treatment of disease, and more particularly, for the treatment of cancer, the method comprising administering a therapeutically effective amount of the pharmaceutical composition of the invention.

In one aspect, the present invention encompasses pharmaceutical compositions, combinations and methods for treating human carcinomas, tumors, as defined above. For example, the invention includes pharmaceutical compositions comprising a pharmaceutically effective amount of an antibody of the present invention and a pharmaceutically acceptable carrier for use in the treatment of human carcinomas.

The bispecific antibody compositions of the invention can be administered using conventional modes of administration including, but not limited to, intravenous, intraperitoneal, oral,

intralymphatic or direct intratumoral administration. Intravenous administration or subcutaneous administration are preferred.

In one aspect of the invention, therapeutic formulations containing the bispecific antibodies of the invention are prepared for storage by mixing an antibody having the desired degree of purity with optional pharmaceutically acceptable carriers, excipients or stabilizers (Remington's Pharmaceutical Sciences 16th edition, Osol, A. Ed. (1980)), in the form of lyophilized formulations or liquid formulations. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed. The formulations to be used for in vivo administration must be sterile. This is readily accomplished by filtration through sterile filtration membranes. The most effective mode of administration and dosage regimen for the pharmaceutical compositions of this invention depends upon the severity and course of the disease, the patient's condition and response to treatment and the judgment of the treating physician. Accordingly, the dosages of the compositions may be flat doses or may be adapted to the individual patient, e.g. the body weight. Nevertheless, an effective dose of the compositions of this invention will generally be in a range from 0.1 to 20 mg/kg.

The bispecific antibodies of this invention have a molecular weight in a magnitude of 150kD per Mol. They carry in one embodiment a Fc part. The elimination half-life in patients is in a range of 3 to 14 days. This half-life allows for, but not limited to administration once a day, once a week, or once every two weeks.

The bispecific antibodies of the present invention and their respective compositions may be in a variety of dosage forms which include, but are not limited to, liquid solutions or suspensions, tablets, pills, powders, suppositories, polymeric microcapsules or microvesicles, liposomes, and injectable or infusible solutions. The preferred form depends upon the mode of administration and the therapeutic application.

The composition comprising a bispecific antibody of the present invention will be formulated, dosed, and administered in a fashion consistent with good medical practice. Factors for consideration in this context include the particular disease or disorder being treated, the particular mammal being treated, the clinic condition of the individual patient, the cause of the disease or

disorder, the site of delivery of the agent, the method of administration, the scheduling of administration, and other factors known to medical practitioners.

Articles of Manufacture

In another aspect of the invention, an article of manufacture containing materials useful for the treatment, prevention and/or diagnosis of the disorders described above is provided. The article of manufacture comprises a container and a label or package insert on or associated with the container. Suitable containers include, for example, bottles, vials, syringes, IV solution bags, etc. The containers may be formed from a variety of materials such as glass or plastic. The container holds a composition which is by itself or combined with another composition effective for treating, preventing and/or diagnosing the condition and may have a sterile access port (for example the container may be an intravenous solution bag or a vial having a stopper pierceable by a hypodermic injection needle). One active agent in the composition is a bispecific antibody of the invention. The label or package insert indicates that the composition is used for treating the condition of choice. Moreover, the article of manufacture may comprise (a) a first container with a composition contained therein, wherein the composition comprises a bispecific antibody of the invention; and (b) a second container with a composition contained therein, wherein the composition comprises a further cytotoxic or otherwise therapeutic agent. The article of manufacture in this embodiment of the invention may further comprise a package insert indicating that the compositions can be used to treat a particular condition. Alternatively, or additionally, the article of manufacture may further comprise a second (or third) container comprising a pharmaceutically-acceptable buffer, such as bacteriostatic water for injection (BWFI), phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user standpoint, including other buffers, diluents, filters, needles, and syringes.

Table 1

SEQUENCE LIST

Sequence Number	Relates to
SEQ ID NO: 1	Common VH

SEQ ID NO: 2	Common CDRH1
SEQ ID NO: 3	Common CDRH2
SEQ ID NO: 4	Common CDRH3
SEQ ID NO: 5	huCD3 VL 1B6
SEQ ID NO: 6	huCD3 1B6 CDRL1
SEQ ID NO: 7	huCD3 1B6 CDRL2
SEQ ID NO: 8	huCD3 1B6 CDRL3
SEQ ID NO: 9	huCD3 VL 1A10
SEQ ID NO: 10	huCD3 1A10 CDRL1
SEQ ID NO: 11	huCD3 1A10 CDRL2
SEQ ID NO: 12	huCD3 1A10 CDRL3
SEQ ID NO: 13	huCD3 VL 1F8
SEQ ID NO: 14	huCD3 1F8 CDRL1
SEQ ID NO: 15	huCD3 1F8 CDRL2
SEQ ID NO: 16	huCD3 1F8 CDRL3
SEQ ID NO: 17	huCD3 VL 1A4
SEQ ID NO: 18	huCD3 1A4 CDRL1
SEQ ID NO: 19	huCD3 1A4 CDRL2
SEQ ID NO: 20	huCD3 1A4 CDRL3
SEQ ID NO: 21	huCD3 VL 1H4
SEQ ID NO: 22	huCD3 1H4 CDRL1
SEQ ID NO: 23	huCD3 1H4 CDRL2
SEQ ID NO: 24	huCD3 1H4 CDRL3
SEQ ID NO: 25	huCD3 1B6 LC
SEQ ID NO: 26	huCD3 1A10 LC

SEQ ID NO: 27	huCD3 1F8 LC
SEQ ID NO: 28	huCD3 1A4 LC
SEQ ID NO: 29	huCD3 1H4 LC
SEQ ID NO: 30	Common constant heavy chain (WT IgG1)
SEQ ID NO: 31	CEA VL AB1 (2F2)
SEQ ID NO: 32	CEA AB1 CDRL1
SEQ ID NO: 33	CEA AB1 CDRL2
SEQ ID NO: 34	CEA AB1 CDRL3
SEQ ID NO: 35	CEA VL AB8 (2A3)
SEQ ID NO: 36	CEA AB8 CDRL1
SEQ ID NO: 37	CEA AB8 CDRL2
SEQ ID NO: 38	CEA AB8 CDRL3
SEQ ID NO: 39	kappa light chain constant region (CK)
SEQ ID NO: 40	CEA AB1 light chain (VKCK_2F2)
SEQ ID NO: 41	lambda light chain constant region (CL)
SEQ ID NO: 42	CEA AB8 light chain (VLCL_2A3)
SEQ ID NO: 43	Common heavy chain (wild-type)
SEQ ID NO: 44	Common heavy chain (LALA mutation)
SEQ ID NO: 45	Common heavy chain (LALA+P329A mutation)
SEQ ID NO: 46	VK_SM3E
SEQ ID NO: 47	VH_SM3E
SEQ ID NO: 48	VL_MEDI
SEQ ID NO: 49	VH_MEDI
SEQ ID NO: 50	VK_SAR
SEQ ID NO: 51	VH_SAR

SEQ ID NO: 52	VK_CH1A1A
SEQ ID NO: 53	VH_ CH1A1A
SEQ ID NO: 54	VK_T84.66
SEQ ID NO: 55	VH_T84.66
SEQ ID NO: 56	VK_LABETUZUMAB
SEQ ID NO: 57	VH_LABETUZUMAB
SEQ ID NO: 58	hybrid-kappa light chain constant region (H-CK 5)
SEQ ID NO: 59	CEA VL 1B4
SEQ ID NO: 60	CEA 1B4 CDRL1
SEQ ID NO: 61	CEA 1B4 CDRL2
SEQ ID NO: 62	CEA 1B4 CDRL3
SEQ ID NO: 63	CEA VL C11
SEQ ID NO: 64	CEA C11 CDRL1
SEQ ID NO: 65	CEA C11 CDRL2
SEQ ID NO: 66	CEA C11 CDRL3
SEQ ID NO: 67	huCD3 1B6 LC-hybrid kappa
SEQ ID NO: 68	huCD3 1A10 LC-hybrid kappa
SEQ ID NO: 69	huCD3 1F8 LC-hybrid kappa
SEQ ID NO: 70	huCD3 1A4 LC-hybrid kappa
SEQ ID NO: 71	huCD3 1H4 LC-hybrid kappa
SEQ ID NO: 72	CEA 2A3 LC-hybrid kappa (VLCK 2A3)
SEQ ID NO: 73	CEA C11 LC (VKCK_C11)
SEQ ID NO: 74	CEA 1B4 LC (VLCL_1B4)
SEQ ID NO: 75	CEA 1B4 LC-hybrid kappa (VLCK_1B4)
SEQ ID NO: 76	VKCK_C11 (DNA)

SEQ ID NO: 77	VLCL_1B4 (DNA)
SEQ ID NO: 78	VKCK_2F2 (AB1) (DNA)
SEQ ID NO: 79	VLCL_2A3 (AB8) (DNA)
SEQ ID NO: 80	Common heavy chain VHCH (wild-type; DNA)
SEQ ID NO: 81	CEA AB13 CDRL1
SEQ ID NO: 82	CEA AB13 CDRL2
SEQ ID NO: 83	CEA AB13 CDRL3
SEQ ID NO: 84	CEA AB14 CDRL1
SEQ ID NO: 85	CEA AB14 CDRL2
SEQ ID NO: 86	CEA AB14 CDRL3
SEQ ID NO: 87	CEA AB15 CDRL1
SEQ ID NO: 88	CEA AB15 CDRL2
SEQ ID NO: 89	CEA AB15 CDRL3
SEQ ID NO: 90	CEA AB17 CDRL1
SEQ ID NO: 91	CEA AB17 CDRL2
SEQ ID NO: 92	CEA AB17 CDRL3
SEQ ID NO: 93	CEA AB20 CDRL1
SEQ ID NO: 94	CEA AB20 CDRL2
SEQ ID NO: 95	CEA AB20 CDRL3
SEQ ID NO: 96	CEA AB54 CDRL1
SEQ ID NO: 97	CEA AB54 CDRL2
SEQ ID NO: 98	CEA AB54 CDRL3
SEQ ID NO: 99	CEA AB60 CDRL1
SEQ ID NO: 100	CEA AB60 CDRL2
SEQ ID NO: 101	CEA AB60 CDRL3

SEQ ID NO: 102	CEA AB66 CDRL1
SEQ ID NO: 103	CEA AB66 CDRL2
SEQ ID NO: 104	CEA AB66 CDRL3
SEQ ID NO: 105	CEA AB71 CDRL1
SEQ ID NO: 106	CEA AB71 CDRL2
SEQ ID NO: 107	CEA AB71 CDRL3
SEQ ID NO: 108	CEA AB72 CDRL1
SEQ ID NO: 109	CEA AB72 CDRL2
SEQ ID NO: 110	CEA AB72 CDRL3
SEQ ID NO: 111	CEA AB73 CDRL1
SEQ ID NO: 112	CEA AB73 CDRL2
SEQ ID NO: 113	CEA AB73 CDRL3
SEQ ID NO: 114	CEA AB13 VL light chain variable region
SEQ ID NO: 115	CEA AB14 VL light chain variable region
SEQ ID NO: 116	CEA AB15 VL light chain variable region
SEQ ID NO: 117	CEA AB17 VL light chain variable region
SEQ ID NO: 118	CEA AB20 VL light chain variable region
SEQ ID NO: 119	CEA AB54 VL light chain variable region
SEQ ID NO: 120	CEA AB60 VL light chain variable region
SEQ ID NO: 121	CEA AB66 VL light chain variable region
SEQ ID NO: 122	CEA AB71 VL light chain variable region
SEQ ID NO: 123	CEA AB72 VL light chain variable region
SEQ ID NO: 124	CEA AB73 VL light chain variable region
SEQ ID NO: 125	CEA AB13 LC light chain
SEQ ID NO: 126	CEA AB14 LC light chain

SEQ ID NO: 127	CEA AB15 LC light chain
SEQ ID NO: 128	CEA AB17 LC light chain
SEQ ID NO: 129	CEA AB20 LC light chain
SEQ ID NO: 130	CEA AB54 LC light chain
SEQ ID NO: 131	CEA AB60 LC light chain
SEQ ID NO: 132	CEA AB66 LC light chain
SEQ ID NO: 133	CEA AB71 LC light chain
SEQ ID NO: 134	CEA AB72 LC light chain
SEQ ID NO: 135	CEA AB73 LC light chain
SEQ ID NO: 136	Consensus light chain CDR1 (XXSQXVXXNLN)
SEQ ID NO: 137	Consensus light chain CDR2 (XXXNRXX)
SEQ ID NO: 138	Consensus light chain CDR3 (QXFXXXXEXNT)

EXAMPLES

Example 1 Cloning, Expression and Purification of Human CEACAM family members

Cloning

The sequence corresponding to the complete extracellular domain (ECD) and A3-B3 domains of CEACAM5 were synthesized and subcloned into the pEAK8 mammalian expression vector (Edge Biosystems, Gaithersburg, Md.). The vectors were modified to introduce an Avitag™ (Avidity, Denver Colo.) and either a hexa-histidine tag, a human FC region or a mouse FC region at the C-terminus. Constructs were verified by DNA sequencing. Purification of recombinant soluble protein was carried out by IMAC (Immobilized Metal Ion Affinity Chromatography), FcXL or CaptureSelect™ IgG-Fc (ms) Affinity Matrix (Thermo Fisher Scientific).

Vectors encoding for the full-length version of human CEACAM 1, 3, 4, 5, 6, 7, 8, 18, 19, 20, 21 and cynomolgus CEACAM5 and CEACAM6 were also generated for expression at the cell surface of PEAK and/or CHO cells. The soluble, full-length human CEACAM16 was also similarly cloned.

Additionally, vectors encoding the following truncated versions of human CEACAM 5 were also generated for expression at the cell surface of PEAK and/or CHO cells: A1-B1-A2-B2-A3-B3; B1-A2-B2-A3-B3; A2-B2-A3-B3; B2-A3-B3; A3-B3. The B3 subdomain is expressed as a fusion protein to the first 140 aa of the human CD86 protein.

Expression

Plasmid mentioned above are then transfected into mammalian cells using a liposome-based transfection reagent such as Lipofectamine2000 (Thermo Fisher Scientific). The transfection step requires only small quantities of DNA and cells, typically $4x10^5$ cells and 2 µg of plasmid DNA per well and the transfection carried out in a 6-well plate. Although different mammalian cell lines can be used, in the examples given below, transformed human embryo kidney monolayer epithelial cells (PEAK cells) are transfected. These cells stably express the EBNA-1 gene, further supporting the episomal replication process, are semi-adherent and can be grown under standard cell culture conditions (5% CO_2; 37°C in DMEM medium supplemented with 10% fetal calf serum). After 24 h, cells are placed under selective conditions by adding medium containing 0.5-2 µg/mL puromycin: cells harboring the episomal vector are resistant to this antibiotic.

Two to three weeks after transfection, amplified and selected cells were injected in disposable CELLine™ bioreactors (Sigma Aldrich) for the production step. The CELLine™ is a two-compartment bioreactor that can be used in a standard cell culture incubator. The smaller compartment (15 ml) contains the cells and is separated from a larger (one liter) medium containing compartment by a semi-permeable membrane with a cut-off size of 10 kDa (Bruce et al. 2002, McDonald et al. 2005). This system allows for the diffusion of nutrients, gazes and metabolic waste products, while retaining cells and secreted proteins in the smaller compartment. The culture is maintained for 7-10 days before harvest of the supernatant. As the medium contains serum, the cells maintain good viability and several production runs can be generated using the same cells and containers.

Purification

After harvest, the cell culture supernatants are clarified by centrifugation. The supernatant is then supplemented with 100 mM imidazole and loaded on Ni-NTA affinity chromatography resin

(Qiagen). The relatively high concentration of imidazole minimizes binding of contaminants to the resin. After washing of the column, proteins are eluted at a flow rate of 2 mL/min using a 30 mL imidazole gradient (20-400 mM imidazole) on an AKTA Prime chromatography system (Cytiva). The elution gradient further improves the purity of the recombinant protein but can be replaced by a step elution approach if a chromatography system is not available. The eluted fractions can be analyzed by SDS-PAGE or ELISA to determine their content in recombinant protein. The fractions of interest are pooled and desalted on Amicon® 10kDa columns (Millipore) equilibrated with phosphate buffered saline or another appropriate buffer. The desalted proteins can then be quantified using various techniques and their purity analyzed by SDS-PAGE. Recombinant proteins are biotinylated in vitro using biotin ligase (Avidity, Denver Colo.) according to manufacturer's instructions. After desalting, the biotinylation level is evaluated by pull-down assays using streptavidin magnetic beads and SDS-PAGE analysis.

Example 2 Phage Display Selection of CEACAM5 Fvs Using Human scFv Libraries Containing Fixed Variable heavy domain

General procedures for construction and handling of human scFv libraries displayed on M13 bacteriophage are described in Vaughan et al., (Nat. Biotech. 1996, 14:309-314), hereby incorporated by reference in its entirety. The libraries for selection and screening encode scFv that all share the same VH domain and are solely diversified in the VL domain. Methods for the generation of fixed VH libraries and their use for the identification and assembly of bispecific antibodies are described in US 2012/0184716 and WO 2012/023053, each of which is hereby incorporated by reference in its entirety. The procedures to identify scFv binding to human CEACAM5 are described below.

Protein Selections

Aliquots of scFv phage libraries (10^{12} Pfu) are blocked with PBS containing 3% (w/v) skimmed milk for one hour at room temperature on a rotary mixer. Blocked phages are deselected on streptavidin magnetic beads (Dynabeads™ M-280) for one hour at room temperature on a rotary mixer. Deselected phages are incubated with 100 nM of either biotinylated human CEACAM5 or the A3-B3 domain captured on streptavidin magnetic beads for two hours at room temperature on a rotary mixer. Beads are captured using a magnetic stand followed by five washes with PBS/0.1% Tween® 20 and two washes with PBS. Phages are eluted with 100 nM TEA for 30

minutes at room temperature on a rotary mixer. Eluted phages and beads are neutralized with Tris-HCl 1M pH 7.4 and directly added to 10 ml of exponentially growing TG1 cells (E.coli strains commonly used in phage display) and incubated for one hour at 37°C with slow shaking (90 rpm). An aliquot of the infected TG1 is serial diluted to titer the selection output. The remaining infected TG1 are spun at 3800 rpm for 10 minutes and resuspended in 2 ml 2xTY and spread on 2xTYAG (2xTY medium containing 100 µg/ml ampicillin and 2% glucose) agar Bioassay plates. After overnight incubation at 30°C, 10 ml of 2xTY is added to the plates and the cells are scraped from the surface and transferred to a 50 ml polypropylene tube. 50% glycerol solution is added to the cell suspension to obtain a final concentration of 17% glycerol. Aliquots of the selection rounds are kept at -80°C.

Phage Rescue

50 µl of cell suspension obtained from previous selection rounds are added to 50 ml of 2xTYAG and grown at 37°C with agitation (240 rpm) until an OD_{600} of 0.3 to 0.5 is reached. The culture is then super-infected with $1.2x10^{11}$ M13K07 helper phage and incubated for one hour at 37°C (90 rpm). The medium is changed by centrifuging the cells at 3800 rpm for 10 minutes, removing the medium and resuspending the pellet in 50 ml of 2xTYAK (2xTY medium containing 100 µg/ml ampicillin; 50 µg/ml kanamycin). The culture is then grown overnight at 30°C (240 rpm). The next day, the phage containing supernatant is used for the next round of selection.

Cell Surface Selections

Phage containing supernatants are blocked with PBS containing 3% (w/v) skimmed milk for one hour at room temperature on a rotary mixer. Blocked phages are then deselected for one hour on MKN-45 $CEACAM5^{KO}$ cells that do not express human CEACAM5. Deselected phages are incubated with $2x10^7$ MKN-45 cells expressing CEACAM5 (blocked in PBS, 3% BSA 0.1% NaN_3) for two hours at room temperature with gentle shaking. Cells are pelleted and washed six times with PBS. Bound phages are eluted with 76 mM citric acid and shaking for 10 minutes. After neutralization with Tris-HCl 1M pH 8 the cells are added directly to 10 ml of exponentially growing TG1 and incubated for one hour at 37°C with slow shaking. An aliquot of the infected TG1 is serial diluted to titer the selection output. Infected TG1 are spun at 3800 rpm for 10 minutes and resuspended in 2 ml 2xTY medium and spread on a 2xTYAG agar Bioassay plate. After overnight incubation at 30°C 10 ml of 2xTY is added to the plate and the cells are scraped

from the surface and transferred to a 50 ml polypropylene tube. 50% glycerol solution is added to the cell suspension to obtain a final concentration of 17% glycerol. Aliquots of the selection rounds are kept at -80°C.

Example 3 Screening for scFv Binding/Non-binding to Soluble CEACAM5, CEACAM6, and CEACAM1

scFv Periplasmic Preparation for Binding and Functional Tests

Individual transformed TG1 clones from selection outputs are inoculated into a deep-well microtiter plate containing 0.9 ml per well of 2xTYAG medium (2xTY medium containing 100 µg/ml ampicillin 0.1% glucose) and grown at 37°C for 5-6 hours (240 rpm). 100 µl per well of 0.2 mM IPTG in 2xTY medium are then added to give a final concentration of 0.02 mM IPTG. The plate is incubated overnight at 30°C with shaking at 240 rpm. The deep-well plate is centrifuged at 3200 rpm for 10 minutes at 4°C and the supernatant carefully removed. The pellets are resuspended in 150 µl TES buffer (50 mM Tris-HCl (pH 8), 1 mM EDTA (pH 8), 20% sucrose, complemented with Complete protease inhibitor, Roche). A hypotonic shock is produced by adding 150 µl of diluted TES buffer (1:5 TES:water dilution) and incubation on ice for 30 minutes. The plate is centrifuged at 4000 rpm for 10 minutes at 4°C to pellet cells and debris. The supernatants are carefully transferred into another microtiter plate and kept on ice for immediate testing in functional assays or binding assays.

Binding

Screening of scFv for binding to CEACAM5 is tested in a homogenous assay using CellInsight™ technology. The following reagents are mixed in each well of a 384 clear bottom well plate (Corning): 30 µl of a streptavidin polystyrene bead suspension (Polysciences; 3000 beads/well) coated with either biotinylated CEACAM5, biotinylated domain A3-B3 or biotinylated NusA for a control protein; 60 µl of blocked scFv periplasmic preparation; 10 µl of detection buffer (PBS containing mouse anti-c-myc antibody at 5 µg/ml; anti-mouse Fc AlexaFluor® 647 diluted 1:200). After mixing at 600 rpm for 5 minutes, the 384-well plate is incubated at room temperature and read after 2 hours on a CellInsight™ CX5 High-Content Screening platform (ThermoFisher Scientific). Clones expressing scFv giving a specific signal for CEACAM5 and not NusA are selected for further analysis or sequencing.

Binding to CEACAM1, CEACAM6 and other CEACAMs can be measured in the same manner.

Clone Sequencing

Single clones are inoculated into a 96-deep-well microtiter plate containing 1 ml LBAG medium (LB medium with 100 µg/ml ampicillin and 2% glucose) per well and grown overnight at 37°C, 300 rpm. DNA is extracted using the Zyppy-96 Plasmid Miniprep kit (Zymo Research) and sequenced.

Example 4 Fixed VH Candidates Reformatting into IgG and Transient Expression in Mammalian Cells

After screening and sequencing, scFv candidates with the desired binding properties are reformatted into IgG and expressed by transient transfection into PEAK cells. The VH and VL sequences of selected scFv are amplified with specific oligonucleotides and cloned into an expression vector containing the heavy and light chain constant regions and the constructions are verified by sequencing. The expression vectors are transfected into mammalian cells using Lipofectamine 2000 (Thermo Fisher Scientific) according to manufacturer's instructions. Briefly, $4x10^6$ PEAK cells are cultured in T75 flasks in 25 ml culture media containing fetal bovine serum. Transfected cells are cultured for 5-6 days at 37°C, IgG production is quantified using an Octet RED96 instrument. The supernatant is harvested for IgG purification on FcXL affinity resin (Thermo Fisher Scientific) according to manufacturer's instructions. Briefly, supernatants from transfected cells are incubated overnight at 4°C with an appropriate amount of FcXL resin. After resin wash with PBS, samples are loaded on Amicon Pro column and the IgG consequently eluted in 50 mM Glycine pH3.5. The eluted IgG fraction is then dialyzed by Amicon 50kDa against Histidine NaCl pH6.0 buffer and the IgG content is quantified by absorption at 280 nm. Purity and IgG integrity are verified by electrophoresis using an Agilent Bioanalyzer 2100 according to manufacturer instructions (Agilent Technologies, Santa Clara, Calif., USA).

Example 5 Characterization of CEACAM5 Monoclonal Antibodies

a) Binding of anti-CEACAM5 arms to cells transfected with different members of the CEACAM family

The specificity of anti-CEACAM5 antibody arms (tested as bivalent mAbs or monovalent bsAbs is shown by flow cytometry using PEAK and/or CHO cells transfected with different members of the CEACAM family.

Vectors encoding the full-length version of human CEACAM 1, 3, 4, 5, 6, 7, 8, 18, 19, 20 and 21 and 20 are used to express these proteins at the surface of PEAK and/or CHO cells as described in Example 1. Similarly, vectors encoding the full-length version of cyno CEACAM 5 and 6 are also used to express these proteins at the surface of PEAK and/or CHO cells. Non-transfected PEAK and/or CHO cells are used as negative control. Cells are harvested, counted, checked for viability and resuspended at 3×10^6 cells/ml in FACS buffer (PBS 2% BSA, 0.1% NaN_3). 100 µl of the cell suspension are distributed in V-bottom 96-well plates (3×10^5 cells/well). The supernatant is removed by centrifugation 3 minutes at 4°C, 1300 rpm and the cells incubated for 15 minutes at 4°C with increasing concentrations of the antibody according to the invention. The antibodies carrying the anti-CEACAM5 arms to test are diluted in FACS buffer and the concentration range is 30 pM-500 nM. Cells are washed twice with cold FACS buffer and re-incubated for further 15 minutes at 4°C with a compatible anti-human IgG secondary antibody. Cells are washed twice with cold FACS buffer and resuspended in 300 µl FACS buffer with 1:1500-diluted TOPRO-3 (Invitrogen). Fluorescence is measured using a FACSCalibur™ (BD Biosciences) or a Cytoflex Platform (Beckman Coulter). Dose-response binding curves are fitted using GraphPad Prism8 software. In the same manner, CEACAM1, CEACAM6 and other CEACAMs can be characterized.

Results obtained by using the experimental procedures described in Examples 1 and 5a are shown in table 2 and 3 (at 10 mcg/ml of the full-size antibodies tested; the BiTE MEDI-565 was tested at equimolar concentration). For the bispecific antibodies AB17L3-1/N, AB71L3-1/N, AB72L3-1/N, AB73L3-1/N the MFI measured for binding to CEACAM5 transfected cells was found between 29000 and 41000 (Table 2). In contrast MFI found by using PEAK cells transfected with CEACAM 1,3,4,6,8 were found below 1000 with the only exception of a strong signal for AB72L3-1/N on CEACAM8 transfected cells. When MFI values obtained on transfected cells expressing any given CEACAM are divided by the values obtained on WT PEAK cells, “factors to PEAK WT” can be calculated (Table 3). With the exception of AB72L3-1/N, the antibodies of the invention are all specific to CEACAM5, as the “factor to PEAK WT” values are all below 2.0. In contrast the MEDI-565 BiTE shows a "factor to PEAK WT” higher than 2 for CEACAM8, suggesting cross-reactivity for such CEACAM family member. This

could cause killing of e.g. neutrophils because as mentioned already above human neutrophils express CEACAM8 on the surface.

TABLE 2 Binding to CEACAMx transiently-expressed on PEAK cells [MFI]

Binding MFI	PEAK WT	CEACAM5	CEACAM1	CEACAM3	CEACAM4	CEACAM6	CEACAM8
AB1L3-1/N	461	7746	441	358	330	373	343
AB17L3-1/N	530	29209	652	633	431	541	411
AB71L3-1/N	440	35076	451	358	323	420	391
AB72L3-1/N	481	41029	652	474	402	562	6289
AB73L3-1/N	512	36240	542	465	352	490	401
MEDI-565 BiTE	1175	18414	1468	943	1338	1829	24002
Y4L3-1/N	253	396	283	298	283	275	249

TABLE 3 Binding to CEACAMx transiently-expressed on PEAK cells [Factor to PEAK WT]

Factor to PEAK WT	PEAK WT	CEACAM5	CEACAM1	CEACAM3	CEACAM4	CEACAM6	CEACAM8
AB1L3-1/N	1.0	16.8	1.0	0.8	0.7	0.8	0.7
AB17L3-1/N	1.0	55.1	1.2	1.2	0.8	1.0	0.8
AB71L3-1/N	1.0	79.7	1.0	0.8	0.7	1.0	0.9
AB72L3-1/N	1.0	85.3	1.4	1.0	0.8	1.2	**13.1**
AB73L3-1/N	1.0	70.8	1.1	0.9	0.7	1.0	0.8
MEDI-565 BiTE	1.0	15.7	1.2	0.8	1.1	1.6	**20.4**
Y4L3-1/N	1.0	1.6	1.1	1.2	1.1	1.1	1.0

b) Binding of CEACAM5 monoclonal antibodies to recombinant proteins in enzyme-linked immunosorbent assay (ELISA)

Biotinylated recombinant human CEACAM5 protein is captured at 0.5 µg/mL in a streptavidin coated 96-well microplate. The plate is washed and monoclonal anti-TAA bivalent antibodies of the present invention are added as a broad concentration-range (e.g. from $5x10^{-4}$ to 1 µg/mL) and incubated during 1 hr. The plate is washed and bound antibodies are detected with an anti-human IgG(Fc)-HRP (Jackson ImmunoResearch). After washing, the plate is revealed with Amplex Red® reagent (Molecular Probes). The fluorescence signal is measured on a Synergy HT plate reader (Biotek).

The binding to other recombinant CEACAM family members such as CEACAM1 and CEACAM6 can be assessed similarly. Binding results are shown in Figures 8 and 9, for the 1B4 mAb and the C11 mAb, respectively.

c) Epitope binning of the antibodies of the invention by competition with reference antibodies

Epitope binning is a competitive immunoassay used to, for example, characterize the binding of new monoclonal antibodies against a target protein. A competitive blocking profile of a new antibody binding to the target protein is created against antibodies also binding to this target protein and for which the binding epitope has already been established/published. Competition to one of these reference antibodies indicate that the new antibody has the same or a closely located epitope and they are "binned" together.

The ability of CEACAM5 mAbs of the present invention to compete with CEACAM5 reference antibodies is tested by ELISA on recombinant human CEACAM5 with the following reference antibodies carrying a mouse Fc region: SM3E, mAb derived from sm3E described in patent US20050147614A1; MEDI, mAb derived from MEDI-565 described in patent WO2016036678A1; SAR, mAb derived from Mab2_VLg5VHg2 described in patent EP3199552A1; CH1A1A, mAb derived from CH1A1A-2F1 described in patent US20120251529 and by Klein *et al* in Oncoimmunology, 2017 Jan 11;6(3); humanized T84.66, mAb derived from variant 1 described in patent WO2017055389; LAB, mAb derived from hMN14 described in patent US 2002/0165360 A1.

SM3E binds e.g. more to the N-terminal, cell membrane distal part of CEA, MEDI to the middle part and CH1A1A binds close to the membrane.

Biotinylated human CEACAM5 is coated at 0.5 µg/ml in a Streptavidin-coated 96-well plate and incubated with 10 µg/ml of the reference mAbs or an irrelevant mAb carrying a mouse Fc region for 1 hour. The CEACAM5 mAbs of the present invention (that means the bivalent monoclonal anti-CEA antibodies) are added at 0.2 µg/ml for 1 hour at room temperature. The plate is washed and the bound CEACAM5 mAbs are detected with an anti-human IgG(Fc)-HRP (Jackson ImmunoResearch). After washing, the plate is revealed with Amplex® Red reagent. The fluorescence signal is measured on a Synergy HT plate reader (Biotek).

Based on the results found with the CEACAM5 mAbs, the derived CEAxCD3 bsAbs according to the invention are considered as competitive with a reference antibody in case binding to CEACAM5 is reduced by more than 80% if the results with and w/o addition of a tool antibody are compared. A CEAxCD3 antibody is identified as non-competitive with a tool antibody in case binding to CEACAM5 is reduced by less than 20% if the results with and w/o addition of a tool antibody are compared. Figure 1 shows in a schematic way the binding regions of reference antibodies used in Examples 5c.

d) Determination of the CEACAM5 domain bound by the antibodies of the invention using truncated form of CEACAM5

Using truncated forms of CEACAM5 lacking one or more of its extracellular subdomains, one could determine the subdomain to which the antibodies of the invention bind.

Vectors encoding the full-length version of human CEACAM5 (containing all its extracellular domains, and namely N-A1-B1-A2-B2-A3-B3) as well as vectors encoding only for a subset of the extracellular domains of CEACAM5 (A1-B1-A2-B2-A3-B3; B1-A2-B2-A3-B3; A2-B2-A3-B3; B2-A3-B3; A3-B3 and B3) are used to express these proteins at the surface of PEAK and/or CHO cells as described in Example 1. Non-transfected PEAK and/or CHO cells are used as negative control. Flow cytometry staining and acquisition is performed as described in example 5, subsection a).

An antibody according to the invention is found as binding to a given truncated CEACAM5 protein, if bound antibody is detected by the PE-conjugated anti-human IgG Fc secondary antibody.

Example 6 Expression and Purification of Bispecific Antibodies Carrying a Lambda and a Kappa Light Chain

The simultaneous expression of one heavy chain and two lights chain in the same cell can lead to the assembly of three different antibodies. Simultaneous expression can be achieved in different ways such as that the transfection of multiple vectors expressing one of the chains to be co-expressed or by using vectors that drive multiple gene expression. The vector encoding the different anti-CEACAM5 antibodies are co-transfected with another vector expressing the heavy and light chain of an anti-CD3 antibody. Alternatively the two light chains are cloned into the vector pNovi κHλ that is previously generated to allow for the co-expression of one heavy chain, one Kappa light chain and one Lambda light chain as described in US 2012/0184716 and WO 2012/023053, each of which is hereby incorporated by reference in its entirety. The expression of the three genes is driven by human cytomegalovirus promoters (hCMV) and the vector also contains a glutamine synthetase gene (GS) that enables the selection and establishment of stable cell lines. The common VH and the VL genes of the anti- CEACAM5 IgG and of the anti-CD3 IgG are cloned in the vector pNovi κHλ, for transient expression in mammalian cells. Expi293 cells are cultured in suspension in an appropriate Erlenmeyer flask with suitable number of cells and culture medium volume. Plasmid DNA is finally transfected into Expi293 cells using PEI. Antibody concentration in the supernatant of transfected cells is measured during the production using an Octet RED96. According to antibody concentration, supernatants are harvested 5 to 7 days after transfection and clarified by centrifugation at 1300 g for 10 min. The purification is based on a three-step purification process. First, the CaptureSelect™ FcXL affinity matrix (Thermo Fisher Scientific) is washed with PBS and then added in the clarified supernatant. After incubation overnight at +4°C and 20 rpm, supernatants are centrifuged at 2000 g for 10 min, flow through is stored and resin washed twice with PBS. Then, the resin is transferred on Amicon Pro columns and a solution containing 50 mM glycine at pH 3.0 is used for elution. Several elution fractions are generated, neutralized with Tris-HCl pH7.4 and pooled. The pool containing total human IgGs (the bispecific and the two monospecific antibodies) is quantified using a Nanodrop spectrophotometer (NanoDrop Technologies, Wilmington, Del.) and then incubated for 30 min at

RT and 20 rpm with the appropriate volume of CaptureSelect™ KappaXL affinity matrix (Thermo Fisher Scientific).GE Healthcare). Resin recovery and wash, elution and neutralization steps are performed as described previously. The last affinity purification step is performed using the CaptureSelect™ lambda Fab affinity matrix (Thermo Fisher Scientific) applying the same process as for the kappa purification step. All elution fractions are pooled and desalted against His-NaCl pH6 formulation buffer using 50kDa Amicon Ultra centrifugal filter units (Merck Millipore). The final product is quantified using the Nanodrop.

Purified bispecific antibodies are analyzed by electrophoresis in denaturing and reducing conditions using The Agilent 2100 Bioanalyzer with the Protein 80 kit as described by the manufacturer (Agilent Technologies, Santa Clara, Calif., USA). 4 µL of purified samples are mixed with sample buffer supplemented with dithiothreitol (DTT; Sigma Aldrich, St. Louis, Mo.). Samples are heated at 95°C for 5 min and then loaded on the chip. All samples are tested for endotoxin contamination using the Limulus Amebocyte Lysate test (LAL; Charles River Laboratories, Wilmington, Mass.).

Example 7: In vitro Characterization of Monovalent and Bispecific Antibodies

a) Binding of Monovalent and Bispecific Antibodies to cells expressing CEACAM5 and cells not expressing CEACAM5

To demonstrate the binding of CD3 x CEACAM5 κλ antibodies to target cells, a series of experiments based on flow cytometry comparing the binding of CD3xCEACAM5 κλ antibodies to their monovalent counterparts can be performed. Examples of cells that can be used include CEACAM5-positive cell lines such as the gastric adenocarcinoma cell line MKN45 (expressing 155'000 CEACAM5 molecules per cell), or the pancreatic adenocarcinoma cell line HPAF-II (expressing 108'000 CEACAM5 molecules per cell) or the colorectal adenocarcinoma cell line LS174T (expressing 26'000 CEACAM5 molecules per cell) and CEACAM5–negative cell lines, such as the lung carcinoma cell line A549 and the MKN45 CEACAM5–knock out cell line generated by CRISPR-CAS9 methodology. Cell staining and binding assessment can be performed as described above. Binding curves obtained are shown in Figures 4, 5, 11 and 14. EC50 values could be calculated using GraphPad Prism8 for the binding to MKN45 cells; data is shown in Table 4. Binding of the bsAb of the invention at 200 nM, 1000 nM and 5000 nM is 40% and higher compared to binding of TCB2014 (see Table 4 and also Figures 11 and 14).

TABLE 4: Binding EC50 to MKN-45 cells. N/A: not applicable

	EC50 (nM)	Top MFI value
AB17L3-1/N	59.2	862'218
AB54L3-1/N	66.4	759'782
AB60L3-1/N	64.1	989'186
AB66L3-1/N	24.3	759'391
AB71L3-1/N	34.1	833'116
AB72L3-1/N	24.5	962'960
AB73L3-1/N	27.8	920'523
Y4L3-1/N	N/A	N/A
TCB2014	11.6	327'315

b) Binding of Monovalent and Bispecific Antibodies to cells expressing CD3 and cells not expressing CD3

To demonstrate the binding of CD3 x CEACAM5 κλ antibodies to effector T-cells, a series of experiments based on flow cytometry comparing the binding of CD3xCEACAM5 κλ antibodies to their monovalent counterparts can be performed. Examples of cells that can be used include human primary T-cells as well as CD3-positive (Jurkat and/or HuT 78) or CD3-negative (TIB-153 and/or JKT-beta-del) cell lines. Cell staining and binding assessment can be performed as described above. Results are shown in Figures 3 and 10.

c) Epitope binning of CEACAM5 antibodies by competition with reference antibodies

Epitope binning is a competitive immunoassay used to characterize the binding of antibodies according to the invention or e.g. the binding of the related anti-CEA (target protein) antibodies of the first binding part. A competitive blocking profile of an antibody binding to the target protein is created against antibodies also binding to this target protein and for which the binding epitope has already been established/published. Competition with one of these reference

antibodies indicate that the antibody has the same or a closely located epitope and they are "binned" together. The ability of anti CEACAM5 arms, which are part of the bispecific antibodies of the present invention to compete with anti CEACAM5 reference antibodies is tested by ELISA with recombinant human CEACAM5 and the following reference antibodies carrying a mouse Fc region: SM3E, sequences of mAb derived from SM3E described in patent US20050147614A1, mAb produced using standard methods; MEDI, mAb derived from MEDI-565 described in patent WO2016036678A1;CH1A1A, mAb derived from CH1A1A-2F1 described in patent US20120251529 and by Klein *et al* in Oncoimmunology, 2017 Jan 11;6(3). SM3E binds more to the N-terminal, cell membrane distal part of CEA, MEDI to the middle part and CH1A1A binds close to the membrane.

κλ bodies, used at 1 μg/ml, are captured by goat anti-human IgG(Fcγ) (Jackson ImmunoResearch) coated at 10 μg/ml on a 96-well black microplate and blocked with Blocking buffer (PBS 2% BSA, 0.05% Tween 20). The competitor IgG (0.03 to 20 μg/ml) are pre-incubated for 1 hour with 0.1 μg/ml Biotinylated human CEACAM5 in Blocking buffer. The κλ body plate is washed and incubated with the pre-incubated competitor IgG/CEACAM5 mixture for 1 hour. After washing the CEACAM5 is detected with Streptavidin-HRP (Jackson ImmunoResearch). The plate is revealed with Amplex™ Red reagent (Molecular Probes) and The fluorescence signal is measured on a Synergy HT plate reader (Biotek).

If the binding of CEACAM5 to the κλ bodies is reduced by the respective tool antibody by 80% or more, it can be concluded that the CEAxCD3 bispecific antibody is classified to bind competitively with the tool antibody. A CEAxCD3 antibody is therefore identified as non-competitive with a tool antibody in case binding of CEACAM5 to the respective κλ body is reduced by 20% or less if the results with and w/o addition of a tool antibody are compared.

d) Binding of Bispecific Antibodies to primary human blood cells

To demonstrate the binding of CD3 x CEACAM5 κλ antibodies to primary T-cells, and the lack of binding to primary B-cells and monocytes (CEA-negative populations), a series of experiments based on flow cytometry can be performed. Cell staining and binding assessment can be performed as described in Example 7a. Data are shown in Figure 13.

Example 8: T-cell Dependent Cellular Cytotoxicity (TDCC) Mediated by Bispecific Antibodies

a) TDCC of CEACAM5 positive and CEACAM5 negative cell lines

The T-cell dependent cellular cytotoxicity (TDCC) of different CEACAM5 positive and CEACAM5 negative tumor cell lines induced by the CEAxCD3 bispecific antibodies of the present invention is assessed using either human PBMCs or purified primary T-cell as effector cells.

Target cells are detached with trypsin or cell dissociation solution after two washes with PBS. After a centrifugation step, cells are resuspended in assay media, adjusted to the needed concentration and plated in 96-well plates.

Effector cells can be either human peripheral blood mononuclear cells (PBMCs) or purified T-cells. PBMCs are isolated from buffy coats derived from healthy human donors using SepMate™ Tubes (Stemcell Technologies) with Lymphoprep™ buffer (Stemcell Technologies). If purified T-cells are used as effector cells, an extra purification step is performed, where T-cells are negatively isolated from PBMCs with the use of a T-cell immunomagnetic negative selection kit (STEMCELL Technologies).

For the TDCC assay, when PBMCs are used as effector cells, these are added to target cells at final E:T ratio of 10: l; when purified T-cells are used, a final E:T ration of 5:1 is used. The CEAxCD3 antibodies of the invention and relevant control antibodies are then added in a dose range concentration (up to 100 nM in duplicates) to the pre-plated target and effector cells. Target cell killing is assessed after either 24h, 48h or 72h of incubation at 37°C, 5% CO_2 by quantifying the LDH released into the medium by apoptotic/necrotic cells (Cytotoxicity Detection KitPLUS (LDH), Roche). Maximal LDH release (= 100% lysis) was obtained by incubating target cells with 1% Triton X-100. Spontaneous LDH release (= 0% lysis) refers to target cells co-incubated with
effector cells without any antibody added. TDCC curves (Figures 6, 7, 12 and 15) and EC50 values (Table 5) can be calculated using GraphPad Prism8 For MKN-45 and LS174T cell lines the EC50 found with the bsAb of the invention shown in Table 5 are significantly lower than the

EC50 measured with TCB2014, demonstrating higher potency for in vitro tumor cell killing of these bsAb of the invention.

TABLE 5 Killing EC50 of three CEA+ cell lines

EC50 (nM)	MKN45	HPAFII	LS174T
AB17L3-1/N	0.11	0.16	0.25
AB54L3-1/N	0.11	0.10	0.13
AB60L3-1/N	0.13	0.28	0.21
AB66L3-1/N	0.05	0.20	0.22
AB71L3-1/N	0.09	0.16	0.16
AB72L3-1/N	0.02	0.12	0.11
AB73L3-1/N	0.03	0.13	0.06
TCB2014	0.85	0.45	1.87
Y4L3-1/N	N/A	N/A	N/A

b) Killing assay by Combination of CEAxCD3 and CEAxCD47 bispecific antibodies

Combinations of a bispecific antibody of this invention with an anti CD47 mAb (such as described in US20140140989 and in WO2017196793) or with a CEAxCD47 bispecific antibody (described in PCT/IB2019/054559, incorporated herein by reference) can be tested in the models described above. Additional test conditions are added to the experimental design, where such CD47-targeting antibody (mono- or bispecific) is used either alone or in combination with a CEAxCD3 antibody of the present invention, at different doses.

c) Up-regulation of T-cell activation markers upon killing of CEA-expressing tumor cells induced by CEAxCD3 bsAbs

Killing of CEA-positive tumor cells induced by CEAxCD3 bsAbs requires T-cell activation, which can be quantified by flow cytometry using antibodies recognizing specific T-cell activation markers such as CD69 (early activation marker) or CD25 (late activation marker).

To assess the activation state of T-cell at the end of a killing assay (described above, example 8a), the following procedure is followed: floating cells (which include both CD4+ and CD8+ T-cells) are transferred in a new V-bottom 96-well plates. The supernatant is removed by centrifugation (3 minutes at 4°C, 1300 rpm) and cells are washed twice with cold FACS buffer (PBS 2% BSA, 0.1% NaN3) before being incubated for 15 minutes at 4°C with Fc-block reagent (BD Biosciences). After two washing with FACS buffer, cells are incubated for 15 minutes at 4°C with following antibodies (used according to the manufacturer's recommendations): Anti CD45 (V500-conjugated, BD Biosciences), CD69 (FITC-conjugated, Biolegend), CD8 (PerCP-Cy5.5-conjugated, Biolegend), CD25 (PE-conjugated, Biolegend), CD4 (APC-conjugated, ThermoFisher), CD3 (APC-R700-conjugated, BD Biosciences).

Cells are washed twice with cold FACS buffer and resuspended in 200 µl FACS buffer. Fluorescence is measured using a Cytoflex Platform (Beckman Coulter) and data is analyzed using FlowJo™ v10 software (BD Life Sciences). Results are shown in Figure 17.

d) <u>T cell proliferation induced by CEAxCD3 bsAbs molecules</u>

CEAxCD3 bsAbs are analyzed for their capability to induce T cell proliferation upon cross-linkage in the presence of CEA positive tumor target cells. As a negative control, CEA negative malignant cells are used as well. Freshly isolated human PBMCs are adjusted to 1 million cells per mL in warm PBS and stained 0.2 µM carboxylfluorescein diacetate succinimidyl ester (CFSE, ThermoFisher Scientific) in PBS for 15 min at 37°C, washed several times with complete RPMI medium (containing 10% FCS, 2mM L-glutamine, 1mM sodium pyruvate, 10mM HEPES, 50 µM 2-mercaptoethanol and 25 µg/mL gentamicin) and transferred into 96-well plates at $2x10^6$ cells per mL. $0.02x10^6$ target cells are plated per well of a flat-bottom 96-well plate and the different CEAxCD3 bsAbs are added at the indicated concentrations. CFSE-labeled PBMCs are added to obtain a final E:T ratio of 10: 1 and the assay plates are incubated for five days in a humidified incubator at 37°C. On day five, the effector cells are harvested, washed twice with FACS buffer (PBS, 2% BSA, 0.1% NaN3), Cells are then stained with BD Horizon 620 (BD Biosciences, 564996) to exclude dead cells and with anti CD45 (V500-conjugated, BD Biosciences), anti-CD4-APC (ThermoFischer, 17-0049-41) and anti-CD8-PerCP-Cy5.5 (Biolegend, 301032). CFSE staining are analyzed on living $CD4^+$ or $CD8^+$ cells by flow cytometry using a CytoFLEX (Beckman Coulter) and results are evaluated by FlowJo software.

e) Cytokine released in the supernatant upon killing of CEA-expressing tumor cells induced by CEAxCD3 bsAbs

Killing of CEA-positive tumor cells induced by CEAxCD3 bsAbs requires T-cell activation. Upon activation, T-cells can release multiple cytokines which can further act as immunomodulating agents. The capacity of the bispecific antibodies of this invention to induce the release of cytokines by T-cells upon killing of CEA-expressing tumor cells was assessed by quantifying selected cytokines in the supernatant at the end of TDCC assays described in Example 8a. Following 2 days of co-culture of CEA-positive target cells and CD3-positive effector T-cells, the culture supernatants were harvested by centrifugation and stored frozen at -80° until further analysis. Cytokines/enzymes such as Granzyme B, IL2, IL6, IL10, TNFα and IFNγ were quantified using the Mesoscale Discovery Platform by using multiplex kits and results are shown in Figure 16

f) TDCC of CEACAM5 positive cell in the presence of shed CEA

CEA positive tumors are known to shed CEA. Such shed CEA could negatively impact the anti-tumor efficacy of CEA targeting antibodies which do not preferentially bind to membrane-bound CEA. To assess whether the bispecific antibodies of this invention are impacted by shed CEA (sCEA), the T-cell dependent cellular cytotoxicity (TDCC) assay described in Example 8a is carried out in the presence of varying concentration of spiked sCEA (BioRad# PHP282). EC50 values in the presence of sCEA are then compared to the EC50 obtained in the absence of sCEA (Table 6). The EC50 calculated for a given sCEA concentration (0.2, 1 or 1 μg/mL) is then compared to that obtained in the absence of sCEA (0 μg/mL) and expressed as EC50 fold change compared to no shed CEA condition. Such values are reported in table 7.

TABLE 6 EC50 of Killing of LS174T cells in the presence of sCEA

	sCEA μg/mL			
	0	0.2	1	5
AB17L3-1/N	0.02	0.07	0.11	0.25
AB54L3-1/N	0.04	0.03	0.16	0.20
AB60L3-1/N	0.29	0.27	0.24	0.38

AB66L3-1/N	0.11	0.13	0.22	0.33
AB71L3-1/N	0.08	0.04	0.31	0.41
AB72L3-1/N	0.06	0.07	0.14	0.43
AB73L3-1/N	0.04	0.04	0.13	0.32
Y4L3-1/N	N/A	N/A	N/A	N/A
TCB2014	0.37	0.12	3.71	*19.83**
TCB2017	0.16	1.19	7.58	*>100**

** no top plateau*

TABLE 7 EC50 fold change compared to no shed CEA condition (0 µg/mL)

	sCEA µg/mL			
	0	0.2	1	5
AB17L3-1/N	1.0	3.2	5.0	11.0
AB54L3-1/N	1.0	0.9	4.6	5.7
AB60L3-1/N	1.0	0.9	0.8	1.3
AB66L3-1/N	1.0	1.2	2.1	3.1
AB71L3-1/N	1.0	0.6	4.2	5.5
AB72L3-1/N	1.0	1.2	2.4	7.3
AB73L3-1/N	1.0	0.8	2.9	7.4
Y4L3-1/N	N/A	N/A	N/A	N/A
TCB2014	1.0	0.3	10.0	*53.4**
TCB2017	1.0	7.7	48.7	*>642.7**

** no top plateau*

Significant higher shifts of EC50 for tumor cell killing were found if sCEA was added for TCB2014 and TCB2017 compared to the bispecific antibodies of the invention at 1 and 5 µg/mL sCEA. Concentrations of 1 µg/mL and above of sCEA are found in patients with CEA positive tumors. The lower shift due to sCEA of the killing curves of the bsAb of the invention suggest less inhibiting influence of high sCEA levels on the efficacy of the bsAb of the invention compared to TCB2014 and TCB2017.

g) TDCC of CEACAM5-negative primary blood cell populations.

Given the mechanism of action of CEAxCD3 bispecific antibodies, cross-reactivity with other CEACAM could lead to depletion of important circulating healthy cell populations. E.g. cross-reactivity with CEACAM8, which is expressed by neutrophils, could lead to the depletion of such cell populations. To confirm the absence of binding, and therefore of killing of such CEA-negative circulating healthy cell populations, purified primary cells such as neutrophils are used as "target cells" instead of CEA-positive cell lines in the experimental procedure described in Example 8a.

Example 9: Evaluation of anti-tumor activity of CEAxCD3 T-cell retargeting molecule as single agent or in combination therapy with CD47-targeting antibodies, in humanized mouse tumor models

a) Anti-tumor activity of CEAxCD3 molecules in PBMC-humanized mouse tumor model

NOG mice (NOD/Shi-scid/OL-2Rγ^{null} mice, Taconic Biosciences), aged of 8-10 weeks, are implanted subcutaneously (s.c.) with 1 to 5x10^6 CEA-positive tumor cells (cell line-derived or patient-derived) and randomized into several treatment groups. Four to seven days later, all the mice are injected i.p. or i.v. with 10 or 20x10^6 of human PBMC (peripheral blood mononuclear cells) for humanization process. CD3xCEA molecules or controls are then administered i.v. starting 3-6 days after PBMC injection, once or twice a week, at different doses. Mice are monitored for tumor development 3 times a week and tumors are measured by digital caliper until the endpoint of the experiment (tumor volume=1500mm^3 or onset of GvHD symptoms). Tumor volume is calculated using the formula (length x $width^2$) x 0.5. Statistical analysis is performed using one-way ANOVA comparison analysis at study termination. Results from an experiment where 1 million HPAF-II cells were engrafted subcutaneously in NOG mice with subsequent injection of 10 million human PBMC are shown in Figure 19.

b) Anti-tumor activity of CEAxCD3 molecules in $CD34^+$-humanized mouse tumor model

Fully humanized $CD34^+$-huNOG mice ($CD34^+$ engrafted NOD/Shi-scid/OL-2Rγ^{null} mice, Taconic Biosciences), aged of 14 weeks and with human $CD45^+$ cells >25% in blood, are implanted subcutaneously (s.c.) with 1 to 5x10^6 CEA-positive tumor cells (cell line-derived or patient-derived) and are randomized into several treatment groups. When the mean tumor volume

reaches a predefined value (ranging from 100 to 200mm^3), CD3xCEA molecules or controls are administered i.v., once or twice a week, at different doses. Mice are monitored for tumor growth 3 times a week and tumors are measured by digital caliper until the endpoint of the experiment (tumor volume=1500mm^3). Tumor volume is calculated using the formula (length x width^2) x 0.5. Statistical analysis is performed using one-way ANOVA comparison analysis at study termination.

c) Anti-tumor activity of CEAxCD3 molecules in combination with CD47-targeting antibodies (monospecific of bispecific) in humanized mouse tumor model

Combinations of a bispecific antibody of this invention with an anti CD47 mAb (such as described in US20140140989 and in WO2017196793) or with a CEAxCD47 bispecific antibody (described in PCT/IB2019/054559, incorporated herein by reference) can be tested in the models described above. Additional groups are added to the experimental design, including treatment groups where a CD47-targeting antibody (monospecific or bispecific) is administered i.v. either alone or in combination with a CEAxCD3 antibody of the present invention, once or twice weekly, at various doses.

d) Anti-tumor activity of CEAxCD3 molecules in combination with CD47-targeting antibodies (monospecific of bispecific) in transgenic mouse tumor models

Combinations of a bispecific antibody of this invention with an anti CD47 mAb (such as described in US20140140989 and in WO2017196793) or with a CEAxCD47 bispecific antibody (described in PCT/IB2019/054559, incorporated herein by reference) can be tested in transgenic mice engineered to express human CD3, human CD47 and human SIRPα, which are implanted subcutaneously (s.c.) with 0.5 to 5x10^6 murine tumor cells engineered to express human CEA and human CD47. When the mean tumor volume reaches a predefined value (ranging from 100 to 200mm^3), mice are randomized. Treatments are administered i.v., once or twice a week, at different doses. Mice are monitored for tumor growth 3 times a week and tumors are measured by digital caliper until the endpoint of the experiment (tumor volume=1500mm^3). Tumor volume is calculated using the formula (length x width^2) x 0.5. Statistical analysis is performed using one-way ANOVA comparison analysis at study termination.

Example 10: Cytokine release tested in whole blood and PBMCs from healthy human donors human blood

An in vitro cytokine release assay is performed using whole blood (WB CRA) with minimal dilution by the test antibodies (95% v/v blood) in aqueous presentation. This assay format is considered to mimic closely the *in vivo* environment, containing factors at physiological concentrations that may influence mechanisms of cytokine release. However, this format is thought to be poorly predictive of T cell-mediated cytokine release (e.g., anti-CD28).

Alternatively, the cytokine release assay can be performed using peripheral blood mononuclear cells (PBMCs) from healthy human donors and with the antibodies in aqueous presentation (Aqueous Phase, AP), to assess T cell-mediated cytokine release (PBMC AP CRA). This format limits cross-linking of mAbs to avoid high cytokine release which is observed with anti-CD3 antibodies upon cross-linking.

Negative controls (anti-EGFR mAb and PBS) as well as specific positive controls (anti-CD52 mAb, CEA x CD3 BiTE and/or anti-CD28 mAb) for each assay format are tested in parallel to the CEA x CD3 bispecific antibodies. After 24 h for WB CRA and 48 h for PBMC AP CRA, supernatants are tested for cytokines in a multiplex assay using electrochemiluminescence as readout (Mesoscale Discovery, Sector 600). IFNγ, TNFα and IL-6 are measured for WB CRA and IFNγ, IL-2, IL-10 and TNFα are measured for PBMC AP CRA. Results are plotted per cytokine with each donor displayed as a single data point.

Example 11 CEA Antibody Affinity Maturation by oligonucleotide-directed mutagenesis using degenerated oligonucleotides (Lead Optimization; LO)

Antibodies identified during the screening process described in the Example 3 are selected for affinity maturation in order to increase their affinity and potency. All these antibodies share the same variable heavy chain but have different variable light chains. AB1 and C11 contain a kappa light chain (IGKV3-11 and IGKV1-5, respectively, according to the IMGT nomenclature) whereas AB8 and 1B4 contain a lambda light chain (IGLV2-14 and IGLV3-21, respectively). Several phage libraries displaying scFv variants are generated by introducing diversity into the CDR1, CDR2 and CDR3 of the variable light chain region while the heavy chain variable region is kept unmodified. Different diversification strategies are used to generate libraries for each candidate, where either the CDRL1+CDRL2; or the CDRL3 only, or all three CDRLs are

diversified (CDRL1+CDRL2+CDRL3) by oligonucleotide-directed mutagenesis of the parental sequence using degenerated oligonucleotides. CDRL1 is diversified in 1 to 5 amino acid positions; CDRL2 in 1 to 4 amino acid positions while CDRL3 in 1 to 5 amino acid positions. A total of up to $5x10^9$ transformants partially covering a theoretical diversity of up to 10^{14} are generated for each candidate.

For candidate AB1 and 1B4, extra libraries diversified in up to 17 amino acid position across all CDRLs are generated with up to $5x10^9$ transformants partially covering a theoretical diversity of up to 10^{21}.

These libraries are used for phage display selections as described in Example 2 except that the selection stringency could be increased between rounds by reducing the concentration of recombinant hCEACAM5 from 100 nM gradually down to 0.01 nM between the different selection rounds or using cells expressing lower levels of hCEACAM5 like the SNUC-1 cell line. The selected variants are screened for the capacity to bind to CEACAM5 using the assay described in Example 3. Positive clones are reformatted as IgG and characterized as described in Examples 4 and 5, respectively.

The anti-CEA arm AB1 (SEQ ID NOs: 31 to 34) was optimized in two successive lead optimization waves. Wave 1 resulted in anti-CEA arms AB13, AB14, AB15, AB17 and AB20. Wave 2 resulted in anti-CEA arms AB54, AB60, AB66, AB71, AB72 and AB73.

Example 12: TDCC (T cell-dependent cellular cytotoxicity) and/or TDCC plus ADCP of tumor derived organoids

Tumor cell derived organoids are an advanced translational model to test T-cell retargeting compounds and/or macrophage and NK cell retargeting compounds.

Organoids are prepared according to standard procedures (Schütte et al., Nature Communications 2017; DOI:10.1038/ncomms14262) and incubated with compounds for up to 8 days in co-culture with PBMCs and in vitro generated macrophages. Medium is changed every 4 days and replaced by fresh medium.

Organoids are collected and enzymatically dissociated into single cells at 37°C for 5 min using Accutase. Cells are pelleted, resuspended in FACS buffer (PBS, 2% FBS, 2 mM EDTA), and filtered through a 400 µm cell strainer. Suspensions of equivalent cell numbers are incubated with antibodies against CD45, CD4, CD8, CEA, and CD14 (all from Thermo Fisher Scientific, Dreieich, Germany) for 30 min on ice. For live-cell gating, propidium iodide is used, measured and analyzed using FlowJo software (FlowJo, LLC, Ashland, OR, USA).

The supernatant from each well is frozen at -80°C for analysis of T-cell activity by using ELISA.

Example 13: TDCC and/or TDCC plus ADCP of patient derived tumor tissue slices

Patient derived fresh tumor tissue slices are another advanced translational model to test T-cell and/or Macrophage and/or NK cell retargeting compounds.

Fresh tumor tissue samples will be cut according to standard procedures as published previously (Sönnichsen et al., Clinical Colorectal Cancer, 17 (2018) e189-e199). In brief, immediately after surgical resection and first macroscopic pathologic assessment, tumor samples are cut into slices of 350 µm using a tissue chopper ((McIlwain TC752; Campden Instruments, Leicestershire, England). Tissue slice diameter is then standardized by using a 3-mm coring tool (kai Europe, Solingen, Germany). Three tissue slices are randomly pooled, placed on membrane inserts, and cultivated in 6-well plates. Slices are incubated under standardized conditions of 37°C and 5% CO_2. Medium is changed 2 hours and 24 hours after preparation prior to treatment.

After 24 hours of pre-cultivation in standard cell culture medium, slice triplets can be exposed to bispecific antibodies according to the invention alone or in combination, respectively, for up to 120 hours. If necessary, incubation time is decreased to 72 hours. Medium will be changed after 72 hours.

After compound exposure, tumor slices are fixed overnight using 4% paraformaldehyde. The supernatant from each well will be frozen at -80°C for analysis of T-cell activity using ELISA.

Paraformaldehyde fixed slices are embedded in paraffin and processed to 5-µm sections. Hematoxylin and eosin (HE) staining is performed to assess histopathologic aspects and tumor

cell proportion. Overall cell count, tumor cell count, and proliferation are analyzed by immunofluorescent staining. In brief, paraffin sections are deparaffinized. After antigen retrieval, sections are washed with 0.3% PBS/TritonX and blocked with 5% normal goat serum (Jackson ImmunoResearch, Suffolk, UK) for 30 minutes. Primary antibodies against cytokeratins (AE1þ3), Ki67, and cleaved-PARP, respectively, are diluted in 0.5% bovine serum albumin and incubated at 4°C overnight. Sections are rinsed with 0.3% phosphate buffered saline/TritonX and labeled with secondary antibodies. Nuclei are stained with Hoechst 33342 (Sigma-Aldrich, St. Louis, MO). For further analysis, antibodies against CEA (tumor cells), CD163 (macrophages), and CD3, CD4, CD8, PD-L1 as well as FoxP3 (all T-cells) are included depending on availability of tumor slices.

Tumor cell-containing area is analyzed in HE sections using slide scans (Pannoramic SCAN and Pannoramic Viewer, 3D Histech, Budapest, Hungary) to investigate varying tumor cell fractions. Slices that contained more benign epithelial cells than neoplastic epithelial cells are excluded from analysis. Slices that did not contain tumor cells are excluded from analysis of proliferating tumor cell fraction but included in analysis of tumor cells per condition. For further analysis, 5 pictures (20x) per tissue slice are taken from fluorescent-stained sections using an Olympus BX51 fluorescent microscope (Olympus Deutschland, Hamburg, Germany). The positive pixel count is determined for Hoechst 33342, cytokeratin, Ki67, and cleaved-PARP stains with stain-specific segmentation algorithms for Image J. Proliferating/apoptotic tumor area is calculated by analyzing pixels of Ki67/cleaved PARP positive nuclei surrounded by cytokeratin-positive pixels.

For every picture, the total cell count (Hoechst-positive), tumor cell count (Hoechst- and cytokeratin-positive), and proliferating tumor cell count (Hoechst-, cytokeratin-, and Ki67-positive/cleavedPARP) is calculated. Tumor cell count is normalized to total cell count and proliferating tumor cell count is normalized to tumor cell count to consider different tumor cell fractions per picture. Mean slice values are then calculated from single image values. Mean values for conditions are calculated using mean slice values.

All publications, patents, patent applications, internet sites, and accession numbers/database sequences including both polynucleotide and polypeptide sequences cited herein are hereby

incorporated by reference herein in their entirety for all purposes to the same extent as if each individual publication, patent, patent application, internet site, or accession number/database sequence were specifically and individually indicated to be so incorporated by reference.

CLAIMS

1. A bispecific antibody comprising a first binding part, specifically binding to human CEACAM5, and a second binding part, specifically binding to human CD3ε, wherein:

a) the first binding part comprises a heavy chain variable region (VH), which comprises a CDRH1 of SEQ ID NO: 2, a CDRH2 of SEQ ID NO: 3 and a CDRH3 of SEQ ID NO: 4,

b) the first binding part comprises a light chain variable region (VL) comprising a CDRL set selected from the group consisting of

b1) a CDRL1 of SEQ ID NO: 90, CDRL2 of SEQ ID NO: 91, and CDRL3 of SEQ ID NO: 92,

b2) a CDRL1 of SEQ ID NO: 96, CDRL2 of SEQ ID NO: 97, and CDRL3 of SEQ ID NO: 98,

b3) a CDRL1 of SEQ ID NO: 99, CDRL2 of SEQ ID NO: 100, and CDRL3 of SEQ ID NO: 101,

b4) a CDRL1 of SEQ ID NO: 102, CDRL2 of SEQ ID NO: 103, and CDRL3 of SEQ ID NO: 104,

b5) a CDRL1 of SEQ ID NO: 105, CDRL2 of SEQ ID NO: 106, and CDRL3 of SEQ ID NO: 107,

b6) a CDRL1 of SEQ ID NO: 108, CDRL2 of SEQ ID NO: 109, and CDRL3 of SEQ ID NO: 110, and

b7) a CDRL1 of SEQ ID NO: 111, CDRL2 of SEQ ID NO: 112, and CDRL3 of SEQ ID NO: 113,

c) the second binding part comprises a VH comprising a CDRH1 of SEQ ID NO: 2, CDRH2 of SEQ ID NO: 3 and CDRH3 of SEQ ID NO: 4, and

d) the second binding part comprises a VL comprising a CDRL1 of SEQ ID NO: 18, CDRL2 of SEQ ID NO: 19, and CDRL3 of SEQ ID NO: 20.

2. The bispecific antibody according to claim 1, comprising

a) in the first binding part a heavy chain variable region VH of SEQ ID NO: 1,

b) in the first binding part a light chain variable region VL selected from the group consisting of

b1) a light chain variable region VL of SEQ ID NO: 117,

b2) a light chain variable region VL of SEQ ID NO: 119,

b3) a light chain variable region VL of SEQ ID NO: 120,

b4) a light chain variable region VL of SEQ ID NO: 121,

b5) a light chain variable region VL of SEQ ID NO: 122,

b6) a light chain variable region VL of SEQ ID NO: 123, and

b7) a light chain variable region VL of SEQ ID NO: 124, and

c) in the second binding part a heavy chain variable region VH of SEQ ID NO: 1 and a light chain variable region VL of SEQ ID NO: 17.

3. The bispecific antibody according to claim 2, comprising

a) in the first binding part a heavy chain variable region VH of SEQ ID NO: 1,

b) in the first binding part a light chain variable region VL selected from the group consisting of

b1) a light chain variable region VL of SEQ ID NO: 117,

b2) a light chain variable region VL of SEQ ID NO: 122, and

b3) a light chain variable region VL of SEQ ID NO: 124, and

c) in the second binding part a heavy chain variable region VH of SEQ ID NO: 1 and a light chain variable region VL of SEQ ID NO: 17.

4. The bispecific antibody according to claim 1, comprising

a) in the first binding part a heavy chain variable region VH of SEQ ID NO: 1,

b) in the first binding part a light chain selected from the group consisting of

b1) the light chain of SEQ ID NO: 128,

b2) the light chain of SEQ ID NO: 130,

b3) the light chain of SEQ ID NO: 131

b4) the light chain of SEQ ID NO: 132,

b5) the light chain of SEQ ID NO: 133,

b6) the light chain of SEQ ID NO: 134, and

b7) the light chain of SEQ ID NO: 135, and

c) in the second binding part a heavy chain variable region VH of SEQ ID NO: 1 and a light chain of SEQ ID NO: 28.

5. The bispecific antibody according to any one of claims 1 to 4, characterized in comprising a common heavy chain selected from the group consisting of

a) the heavy chain of SEQ ID NO: 43,

b) the heavy chain of SEQ ID NO: 44, and

c) the heavy chain of SEQ ID NO: 45.

6. The bispecific antibody according to claim 1 comprising a common heavy chain of SEQ ID NO: 45 and in the second binding part a light chain of SEQ ID NO: 28 and in the first binding part a light chain of SEQ ID NO: 128.

7. The bispecific antibody according to claim 1 comprising a common heavy chain of SEQ ID NO: 45 and in the second binding part a light chain of SEQ ID NO: 28 and in the first binding part a light chain of SEQ ID NO: 133.

8. The bispecific antibody according to claim 1 comprising a common heavy chain of SEQ ID NO: 45 and in the second binding part a light chain of SEQ ID NO: 28 and in the first binding part a light chain of SEQ ID NO: 135.

9. The bispecific antibody according to any one of claims 1 to 8, wherein the bispecific antibody comprises an IgG Fc domain and each subunit of the Fc domain comprises the amino acid substitutions L234A, L235A and P329A (Kabat EU index numbering).

10. Use of the bispecific antibody according to any one of claims 1 to 9 in the manufacture of a medicament for treating colorectal cancer, non-small cell lung cancer (NSCLC), gastric cancer, pancreatic cancer and/or breast cancer that expresses CEA.

11. Use of the bispecific antibody according to any one of claims 1 to 9 in the manufacture of a medicament for treating carcinomas that express CEA.

12. The bispecific antibody according to any one of claims 1 to 9 for use in therapy.

13. Use of the bispecific antibody according to any one of claims 1 to 9 in the manufacture of a medicament for treating colorectal carcinoma, esophageal cancer, pancreatic adenocarcinoma, gastric cancer, non-small cell lung cancer, breast cancer, head and neck carcinoma, uterine and bladder cancers that express CEA.

14. Use of the bispecific antibody according to any one of claims 1 to 9 in the manufacture of a medicament for use as a monotherapy for the treatment of CEA expressing solid tumors.

15. Use of the bispecific antibody according to any one of claims 1 to 9 in the manufacture of a first medicament and use of pembrolizumab, nivolumab, pidilizumab, or lambrolizumab in the manufacture of a second medicament, wherein the first and the second medicament are for use in combination therapy to treat cancer that expresses CEA, wherein the first and the second medicaments are to be administered simultaneously, separately, or sequentially.

16. The bispecific antibody of claim 12 or the use according to any one of claims 10 to 11 and 13 to 15, wherein the medicament is to be administered to a patient in doses ranging from 0.1 to 100 mg/kg of body weight per day or per week in single or divided doses, or by continuous infusion.

17. The use according to claim 15, wherein pembrolizumab, nivolumab, pidilizumab, or lambrolizumab is to be administered to a patient in doses ranging from 0.1 to 100 mg/kg of body weight per day or per week in single or divided doses, or by continuous infusion.

18. A pharmaceutical composition comprising a bispecific antibody according to any one of claims 1 to 9 and a pharmaceutically acceptable carrier.

19. Use of the bispecific antibody of any one of claims 1 to 9, or the pharmaceutical composition of claim 18, in the manufacture of a medicament for treating cancer that expresses CEA in a subject in need thereof.

20. An isolated polynucleotide that encodes the antibody of any one of claims 1 to 9.

21. A vector comprising the isolated polynucleotide of claim 20.

22. A cell comprising the isolated polynucleotide of claim 20 or the vector of claim 21.

23. The cell of claim 22, which is selected from the group consisting of Streptomyces, yeast, CHO, YB/20, NS0, PER-C6, HEK-293T, NIH-3T3, HeLa, BHK, Hep G2, SP2/0, R1.1, B-W, L-M, COS 1, COS 7, BSC1, BSC40, BMT10 cell, plant cell, insect cell, and human cell in tissue culture.

24. A method of making a bispecific antibody, comprising expressing the bispecific antibody in the cell of claim claim 22 or 23.

25. A method of making a bispecific antibody, comprising culturing the cell of claim 22 or 23 and isolating the antibody expressed therein.