



(86) **Date de dépôt PCT/PCT Filing Date:** 2009/10/01
(87) **Date publication PCT/PCT Publication Date:** 2010/04/08
(45) **Date de délivrance/Issue Date:** 2024/04/30
(85) **Entrée phase nationale/National Entry:** 2011/03/25
(86) **N° demande PCT/PCT Application No.:** EP 2009/062794
(87) **N° publication PCT/PCT Publication No.:** 2010/037837
(30) **Priorité/Priority:** 2008/10/01 (US61/101,933)

(51) **Cl.Int./Int.Cl.** **C07K 16/28** (2006.01),
C07K 16/22 (2006.01), **C07K 16/30** (2006.01),
C07K 16/40 (2006.01)
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(54) **Titre : ANTICORPS MONOCATENAIRES BISPECIFIQUES SPECIFIQUES D'ANTIGENES CIBLES DE MASSE MOLECULAIRE ELEVEE**

(54) **Title: BISPECIFIC SINGLE CHAIN ANTIBODIES WITH SPECIFICITY FOR HIGH MOLECULAR WEIGHT TARGET ANTIGENS**

(57) **Abrégé/Abstract:**

The present invention provides a method for the selection of bispecific single chain antibodies comprising a first binding domain capable of binding to an epitope of CD3 and a second binding domain capable of binding to the extracellular domain cell surface antigens with a high molecular weight extracellular domain. Moreover, the invention provides bispecific single chain antibodies produced by the use of the method of the invention, nucleic acid molecules encoding these antibodies, vectors comprising such nucleic acid molecules and methods for the production of the antibodies. Furthermore, the invention provides pharmaceutical compositions comprising bispecific single chain antibodies of the invention, medical uses of the same and methods for the treatment of diseases comprising the administration of bispecific single chain antibodies of the invention.



(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau(43) International Publication Date
8 April 2010 (08.04.2010)

PCT

(10) International Publication Number
WO 2010/037837 A3

(51) International Patent Classification:

C07K 16/28 (2006.01) C07K 16/30 (2006.01)
C07K 16/22 (2006.01) C07K 16/40 (2006.01)

(21) International Application Number:

PCT/EP2009/062794

(22) International Filing Date:

1 October 2009 (01.10.2009)

(25) Filing Language:

English

(26) Publication Language:

English

(30) Priority Data:

61/101,933 1 October 2008 (01.10.2008) US

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(81) Designated States (unless otherwise indicated, for every kind of national protection available): AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ,

CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, RO, RS, RU, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States (unless otherwise indicated, for every kind of regional protection available): ARIPO (BW, GH, GM, KE, LS, MW, MZ, NA, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

Declarations under Rule 4.17:

— as to the applicant's entitlement to claim the priority of the earlier application (Rule 4.17(iii))

Published:

— with international search report (Art. 21(3))
— before the expiration of the time limit for amending the claims and to be republished in the event of receipt of amendments (Rule 48.2(h))

(88) Date of publication of the international search report:

22 July 2010

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WO 2010/037837 A3

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5 **Bispecific single chain antibodies with specificity for high molecular weight
 target antigens**

The present invention provides a method for the selection of bispecific single chain antibodies comprising a first binding domain capable of binding to an epitope of CD3 and a second binding domain capable of binding to to the extracellular domain cell surface antigens with a high molecular weight extracellular domain. Moreover, the
10 invention provides bispecific single chain antibodies produced by the use of the method of the invention, nucleic acid molecules encoding these antibodies, vectors comprising such nucleic acid molecules and methods for the production of the antibodies. Furthermore, the invention provides pharmaceutical compositions comprising bispecific single chain antibodies of the invention, medical uses of the
15 same and methods for the treatment of diseases comprising the administration of bispecific single chain antibodies of the invention.

Unifying two antigen binding sites of different specificity into a single construct, bispecific antibodies have the ability to bring together two discrete antigens with
20 exquisite specificity and therefore have great potential as therapeutic agents. This potential was recognized early on, leading to a number of approaches for obtaining such bispecific antibodies. Bispecific antibodies were originally made by fusing two hybridomas, each capable of producing a different immunoglobulin. The resulting hybrid-hybridoma, or quadroma, was capable of producing antibodies bearing the
25 antigen specificity of the first parent hybridoma as well as that of the second parent hybridoma (Milstein et al., (1983) Nature 305, 537). However, the antibodies resulting from quadromas often exhibited undesired properties due to the presence of an Fc antibody portion.

30 Largely due to such difficulties, attempts later focused on creating antibody constructs resulting from joining two scFv antibody fragments while omitting the Fc portion present in full immunoglobulins. Each scFv unit in such constructs was made up of one variable domain from each of the heavy (VH) and light (VL) antibody chains, joined with one another via a synthetic polypeptide linker, the latter often

being genetically engineered so as to be minimally immunogenic while remaining maximally resistant to proteolysis. Respective scFv units were joined by a number of techniques including incorporation of a short (usually less than 10 amino acids) polypeptide spacer bridging the two scFv units, thereby creating a bispecific single chain antibody. The resulting bispecific single chain antibody is therefore a species containing two VH/VL pairs of different specificity on a single polypeptide chain, wherein the VH and VL domains in a respective scFv unit are separated by a polypeptide linker long enough to allow intramolecular association between these two domains, and wherein the thusly formed scFv units are contiguously tethered to one another through a polypeptide spacer kept short enough to prevent unwanted association between, for example, the VH domain of one scFv unit and the VL of the other scFv unit.

Bispecific single chain antibodies of the general form described above have the advantage that the nucleotide sequence encoding the four V-domains, two linkers and one spacer can be incorporated into a suitable host expression organism under the control of a single promoter. This increases the flexibility with which these constructs can be designed as well as the degree of experimenter control during their production.

Remarkable experimental results have been obtained using such bispecific single chain antibodies designed for the treatment of malignancies (Mack, J. Immunol. (1997) 158, 3965-70; Mack, PNAS (1995) 92, 7021-5; Kufer, Cancer Immunol. Immunother. (1997) 45, 193-7; Löffler, Blood (2000) 95, 2098-103) and non-malignant diseases (Brühl, J. Immunol. (2001) 166, 2420-6); Brischwein et al. J Immunother. (2007) 30(8), 798-807; Bargou, et al. (2008) Science 321, 974). In such bispecific single chain antibodies, one scFv unit is capable of activating cytotoxic cells, for example cytotoxic T cells, within the immune system by specifically binding to an antigen on the cytotoxic cells, while the other scFv unit specifically binds an antigen on a malignant cell intended for destruction. In this way, such bispecific single chain antibodies have been shown to activate and redirect the immune system's cytotoxic potential to the destruction of pathological, especially malignant cells. In the absence of such a bispecific single chain antibody construct, malignant cells would otherwise proliferate uninhibited.

When designing a new bispecific single chain antibodies comprising one scFv unit is capable of recruiting cytotoxic cells, for example cytotoxic T cells, while the other scFv unit specifically binds an antigen on a target cell to be eliminated by the recruited cytotoxic cell, it has been observed that different combination of scFv's in the bispecific single chain antibodies show different effectivity in the elimination of the target cells. The election of a promising candidate is an intensive and time consuming procedure.

- 10 The present invention provides means and methods for the solution of this problem for a bispecific single chain antibodies binding with one domain to cytotoxic cells, i.e. cytotoxic T cells, and with the second binding domain to target antigens with a high molecular weight extracellular domain.
- 15 Accordingly, the present invention provides in a first embodiment a method for the selection of bispecific single chain antibodies comprising a first binding domain capable of binding to an epitope of CD3 and a second binding domain capable of binding to the extracellular domain cell surface antigens with a high molecular weight extracellular domain. Different binding domains, which may be used as first binding
- 20 domain, are described in the art and in the appended sequence listing. As apparent from the above, the election of an antigenic domain on a target cell for of preparation of a target cell binding domain of a bispecific single chain antibody is the critical step for the provision of new bispecific single chain antibodies which allow for an efficient elimination of target cells via redirected T cell lysis. A first choice for the election of
- 25 an antigenic domain on a target cell for of preparation of a target cell binding domain of a bispecific single chain antibody might be a domain, which is easily accessible from a steric point of view. Accordingly, the person skilled in the art would elect in the case cell surface proteins on target cells with a high molecular weight extracellular domain epitopes which are most distant from the target cell surface are most
- 30 exposed, therefore best accessible for T cells and thus particularly potent in redirecting T cell cytotoxicity. However, it has been surprisingly found that membrane distant epitopes of target cell surface antigens with a high molecular weight extracellular domain show a poor potency of redirecting T cell cytotoxicity.

The method of the invention provides guidance for the election of antigenic regions of cell surface antigens with a high molecular weight extracellular domain which allow for the selection of bispecific single chain antibodies with a high potency for redirected T cell cytotoxicity. These cell surface antigens with a high molecular weight extracellular domain are type I or type II integral membrane proteins with an extracellular portion of > 640 amino acids. The extracellular portion of this group of membrane proteins is independently folded, thus formed by a single continuous stretch of extracellular amino acids adjacent to the transmembrane region in the primary protein sequence. In order to fulfil the requirement of a high molecular weight extracellular domain in the context of the invention, the extracellular domain essentially comprises more than 640 amino acids. Optionally the extracellular domain is characterized by at least one functionally and/or structurally defined subdomain formed by discontinuous stretches of extracellular amino acids within the primary protein sequence. Examples for such cell surface antigens comprise prostate-specific membrane antigen (PSMA), fibroblast activation protein α (FAP α), Hepatocyte Growth Factor Receptor (c-MET), endosialin (TEM1 or CD248) and type 1 insulin-like growth factor receptor (IGF-1R).

PSMA and FAP α are cell surface molecules for which the crystal structure and, thus, the three dimensional structure of the extracellular domain are known in the art. These antigens show a compact discontinuous domain composition of the extracellular domain. It has been surprisingly found that bispecific single chain antibodies binding to epitopes with a distance of up to 60Å from the alpha C-atom of the thirteenth extracellular amino acid as counted from the junction of transmembrane and extracellular region (reference C-atom) show a significant high efficiency in the redirected T cell lysis of target cells. In contrast thereto, the efficiency in the redirected T cell lysis of target cells of bispecific single chain antibodies binding only to epitopes with a distance of more than 60Å from the reference C-atom is reduced and thus renders such bispecific antibodies unattractive for a clinical development.

c-MET, TEM1 and IGF-1R are cell surface molecules having a consecutive sequence of independently folded extracellular domains is formed by a corresponding sequence of continuous stretches of extracellular amino acids within the primary protein sequence. It has been surprisingly found that bispecific single chain antibodies binding to epitopes within the first 640 amino acid residues counted

from the junction of transmembrane and extracellular region show a significant high efficiency in the redirected T cell lysis of target cells. In contrast thereto, the efficiency in the redirected T cell lysis of target cells of bispecific single chain antibodies binding only to epitopes within the amino acid residues above the 640th amino acid residue counted from the junction of transmembrane and extracellular region is reduced and thus renders such bispecific antibodies unattractive for a clinical development.

Based on these findings the invention relates in one embodiment to a method for the selection of bispecific single chain antibodies comprising a first binding domain capable of binding to an epitope of CD3 and a second binding domain capable of binding to the extracellular domain of prostate-specific membrane antigen (PSMA), the method comprising the steps of:

- (a) providing at least three types of host cells expressing
 - (i) the wt human extracellular domain of PSMA (SEQ ID NO: 447) on the cell surface;
 - (ii) a mutated form of the wt human PSMA on the cell surface, wherein the amino acid residues at positions 140, 169, 191, 308, 334, 339, 344, 624, 626, 716, 717 and 721 are mutated to the corresponding amino acid residues of the wt rodent PSMA; and
 - (iii) the rodent wt extracellular domain of PSMA on the cell surface;
- (b) contacting each type of host cells (i), (ii) and (iii) of step (a) with the bispecific single chain antibodies and effector T cells; and
- (c) identifying and isolating the bispecific single chain antibodies that mediate the lysis of host cells expressing wt human extracellular domain of PSMA on the cell surface according to (b)(i) and of host cells expressing mutated form of the wt human PSMA on the cell surface according to (b)(ii) but not of host cells expressing the rodent wt extracellular domain of PSMA on the cell surface according to b(iii).

As noted above, prostate-specific membrane antigen (PSMA; PSM) is a large antigen falling under the provided definition of cell surface antigens with a high molecular weight extracellular domain. Israeli et al. (Cancer Res. 53: 227-230, 1993) cloned a 2.65-kb cDNA for a prostate-specific membrane antigen detected with a monoclonal antibody raised against the human prostatic carcinoma cell line LNCaP. The PSMA gene encodes a 750-amino acid protein that has an apparent molecular

weight of 100 kD (due to posttranslational modification) and is expressed by normal and neoplastic prostate cells. PSMA was originally defined by the monoclonal antibody (MAb) 7E11 derived from immunization with a partially purified membrane preparation from the lymph node prostatic adenocarcinoma (LNCaP) cell line
5 (Horoszewicz et al., *Anticancer Res.* 7 (1987), 927-35). A 2.65-kb cDNA fragment encoding the PSMA protein was cloned and subsequently mapped to chromosome 11p11.2 (Israeli et al., loc. cit.; O'Keefe et al., *Biochem. Biophys. Acta* 1443 (1998), 113-127). Initial analysis of PSMA demonstrated widespread expression within the cells of the prostatic secretory epithelium. Immunohistochemical staining
10 demonstrated that PSMA was absent to moderately expressed in hyperplastic and benign tissues, while malignant tissues stained with the greatest intensity (Horoszewicz et al., loc. cit.). Subsequent investigations have recapitulated these results and evinced PSMA expression as a universal feature in practically every prostatic tissue examined to date. These reports further demonstrate that expression
15 of PSMA increases precipitously proportional to tumor aggressiveness (Burger et al., *Int. J. Cancer* 100 (2002), 228-237; Chang et al., *Cancer Res.* 59 (1999), 3192-98; Chang et al., *Urology* 57 (2001), 1179-83), Kawakami and Nakayama, *Cancer Res.* 57 (1997), 2321-24; Liu et al., *Cancer Res.* 57 (1997), 3629-34; Lopes et al., *Cancer Res.* 50 (1990), 6423-29; Silver et al., *Clin. Cancer Res.* 9 (2003), 6357-62; Sweat et al., *Urology* 52 (1998), 637-40; Troyer et al., *Int. J. Cancer* 62 (1995), 552-558;
20 Wright et al., *Urology* 48 (1996), 326-334). Consistent with the correlation between PSMA expression and tumor stage, increased levels of PSMA are associated with androgen-independent prostate cancer (PCa). Analysis of tissue samples from patients with prostate cancer has demonstrated elevated PSMA levels after physical
25 castration or androgen-deprivation therapy. Unlike expression of prostate specific antigen, which is downregulated after androgen ablation, PSMA expression is significantly increased in both primary and metastatic tumor specimens (Kawakami et al., Wright et al., loc. cit.). Consistent with the elevated expression in androgen-independent tumors, PSMA transcription is also known to be downregulated by
30 steroids, and administration of testosterone mediates a dramatic reduction in PSMA protein and mRNA levels (Israeli et al., *Cancer Res.* 54 (1994), 1807-11; Wright et al., loc. cit.). PSMA is also highly expressed in secondary prostatic tumors and occult metastatic disease. Immunohistochemical analysis has revealed relatively intense and homogeneous expression of PSMA within metastatic lesions localized to lymph

nodes, bone, soft tissue, and lungs compared with benign prostatic tissues (Chang et al. (2001), loc. cit.; Murphy et al., Cancer 78 (1996), 809-818; Sweat et al., loc. cit.). Some reports have also indicated limited PSMA expression in extraprostatic tissues, including a subset of renal proximal tubules, some cells of the intestinal brush-border membrane, and rare cells in the colonic crypts (Chang et al. (1999), Horoszewicz et al., Israeli et al. (1994), Lopes et al., Troyer et al., loc. cit.). However, the levels of PSMA in these tissues are generally two to three orders of magnitude less than those observed in the prostate (Sokoloff et al., Prostate 43 (2000), 150-157). PSMA is also expressed in the tumor-associated neovasculature of most solid cancers examined yet is absent in the normal vascular endothelium (Chang et al. (1999), Liu et al., Silver et al., loc. cit.). Although the significance of PSMA expression within the vasculature is unknown, the specificity for tumor-associated endothelium makes PSMA a potential target for the treatment of many forms of malignancy.

As apparent from SEQ ID NO: 447 the extracellular domain of PSMA comprises 707 amino acid residues. The 13th aa as counted from the junction of transmembrane and extracellular region (reference C-atom) is a histidine. The identification of the amino acid residues to be mutated for the mutant human PSMA is described in detail in appended example 2. According to the method of the invention all amino acid residues which do not match between the mouse and the rodent extracellular domain of PSMA and which have a distance of more than 60Å from the reference C-atom are mutated from the human sequence to the rodent sequence. This mutation results in a transformation of all antigenic regions with a distance of more than 60Å from the reference C-atom from the human specific form to the rodent specific form. Antibodies, e.g. bispecific antibodies which are specific for human epitopes comprising antigenic regions with a distance of more than 60Å from the reference C-atom (specific for membrane distal epitopes) do not bind to the mutant human PSMA and the rodent PSMA. Accordingly, the method of the invention allows for a discrimination of antibodies which bind to epitopes comprising antigenic regions with a distance of more than 60Å from the reference C-atom (antibodies specific for membrane distal epitopes) and a positive identification and isolation of antibodies specific for epitopes of the human PSMA within a distance of less than 60Å from the reference C-atom (antibodies specific for membrane proximal epitopes).

As apparent from the appended examples it has been surprisingly observed that the distance of the epitope from the cell membrane is a critical factor for the cytotoxic

potency of a bispecific single chain antibody which engages effector T cells and target cells, such as PSMA⁺ cells. The general effect underlying the distance between the epitope, which is bound by an according bispecific single chain antibody, from the cell membrane of a target cell is exemplified in a model in the
5 appended example 1. What came as a surprise according to this example, however, was the large extent of the loss in target cell lysis observed between a target size of 640 aa (D1) and 679 aa (D3). Despite this small difference in target size there was more loss in target cell lysis than from 679 aa (D3) to 1319 aa (D1 + D3). Thus, a target size of 640 aa was unexpectedly found as upper threshold for the membrane-
10 distant epitopes of bispecific single chain antibodies, still capable of inducing redirected T cell cytotoxicity with reasonable potency without requiring compensation for the negative influence of more membrane-distance by other properties of the bscAb such as a very high affinity to the target antigen. Moreover, the cytotoxic potency relative to the distance of the epitope bound by a bispecific single chain
15 antibody is demonstrated in examples 3 and 4.

Examples for assays for performing the steps of the method according to the invention are described in the appended examples.

The invention further relates to a method for the selection of bispecific single chain
20 antibodies comprising a first binding domain capable of binding to an epitope of CD3 and a second binding domain capable of binding to the extracellular domain of fibroblast activation protein α (FAP α), the method comprising the steps of:

- (a) providing at least three types of host cells expressing
 - (i) the wt human extracellular domain of FAP α (SEQ ID NO: 448) on the cell
25 surface;
 - (ii) a mutated form of the wt human FAP α on the cell surface, wherein the amino acid residues at positions 144, 185, 186, 229, 267, 273, 274, 278, 284, 301, 328, 329, 331, 335 and 362 are mutated to the corresponding amino acid residues of the wt rodent FAP α ; and
30
 - (iii) the rodent wt extracellular domain of FAP α on the cell surface;
- (b) contacting each type of host cells (i), (ii) and (iii) of step (a) with the bispecific single chain antibodies and effector T cells; and
- (c) identifying and isolating the bispecific single chain antibodies that mediate the lysis of host cells expressing wt human extracellular domain of FAP α on the cell

surface according to (b)(i) and of host cells expressing mutated form of the wt human FAP α on the cell surface according to (b)(ii) but not of host cells expressing the rodent wt extracellular domain of FAP α on the cell surface according to b(iii).

- 5 Another large antigen according to the above definition is the cell surface protease fibroblast activation protein alpha (FAP alpha). In epithelial cancer, invasion and metastasis of malignant epithelial cells into normal tissues is accompanied by adaptive changes in the mesenchyme-derived supporting stroma of the target organs. Altered gene expression in these non-transformed stromal cells has been
- 10 discussed to provide potential targets for therapy. FAP alpha is such an example for a target of activated tumor fibroblasts in tumor stroma. Fibroblast activation protein alpha is an inducible cell surface glycoprotein that has originally been identified in cultured fibroblasts using monoclonal antibody F19. Immunohistochemical studies have shown that FAP alpha is transiently expressed in certain normal fetal
- 15 mesenchymal tissues but that normal adult tissues as well as malignant epithelial, neural, and hematopoietic cells are generally FAP alpha-negative. However, most of the common types of epithelial cancers contain abundant FAP alpha-reactive stromal fibroblasts. FAP alpha cDNA was cloned and published in GenBank (Accession number NM_004460). The predicted human FAP alpha protein is a type II integral
- 20 membrane protein with a large C-terminal extracellular domain, which contains 6 potential N-glycosylation sites, 13 cysteine residues, and 3 segments that correspond to highly conserved catalytic domains of serine proteases; a hydrophobic transmembrane segment; and a short cytoplasmic tail. FAP-alpha shows 48% amino acid identity with dipeptidyl peptidase IV (DPP4) and 30% identity with DPP4-related
- 25 protein (DPPX). Northern blot analysis detected a 2.8-kb FAP alpha mRNA in fibroblasts. Seprase is a 170-kD integral membrane gelatinase whose expression correlates with the invasiveness of human melanoma and carcinoma cells. Goldstein et al. (Biochim. Biophys. Acta 1361: 11-19, 1997) cloned and characterized the corresponding seprase cDNA. The authors found that seprase and FAP alpha are
- 30 the same protein and products of the same gene. Pineiro-Sanchez et al. (J. Biol. Chem. 272: 7595-7601, 1997) isolated seprase/FAP alpha protein from the cell membranes and shed vesicles of human melanoma LOX cells. Serine protease inhibitors blocked the gelatinase activity of seprase/FAP alpha, suggesting that seprase/FAP alpha contains a catalytically active serine residue(s). The authors

- found that seprase/FAP alpha is composed of monomeric, N-glycosylated 97-kD subunits that are proteolytically inactive. They concluded that seprase/FAP alpha is similar to DPP4 in that their proteolytic activities are dependent upon subunit association. Due to its degrading activity of gelatine and heat-denatured type-I and type-IV collagen, a role for seprase/FAP alpha in extracellular matrix remodeling, tumor growth, and metastasis of cancers has been suggested. Moreover, seprase/FAP alpha shows a restricted expression pattern in normal tissues and a uniform expression in the supporting stroma of many malignant tumors. Therefore, seprase/FAP alpha may be used as a target for exploring the concept of tumor stroma targeting for immunotherapy of human epithelial cancer. However, though several clinical trials have been initiated to investigate seprase's/FAP alpha's role as a tumor antigen target, conventional immunotherapy approaches or inhibition of seprase/FAP alpha enzymatic activity so far did not yet result in therapeutic efficacy (see e.g. Welt et al., J. Clin. Oncol. 12:1193-203, 1994; Narra et al., Cancer Biol. Ther. 6, 1691-9, 2007; Henry et al., Clinical Cancer Research 13, 1736-1741, 2007).
- As apparent from SEQ ID NO: 448 the extracellular domain of FAP α comprises 734 amino acid residues. The 13th aa as counted from the junction of transmembrane and extracellular region (reference C-atom) is a methionine. The identification of the amino acid residues to be mutated for the mutant human FAP α is described in detail in appended example 5. According to the method of the invention all amino acid residues which do not match between the mouse and the rodent extracellular domain of FAP α and which have a distance of more than 60Å from the reference C-atom are mutated from the human sequence to the rodent sequence.
- Moreover, the invention relates to a method for the selection of bispecific single chain antibodies comprising a first binding domain capable of binding to an epitope of CD3 and a second binding domain capable of binding to the extracellular domain of Hepatocyte Growth Factor Receptor (c-MET), endosialin (TEM1) and type 1 insulin-like growth factor receptor (IGF-1R), the method comprising the steps of:
- (a) identifying the membrane proximal 640 amino acid residues of the human and the rodent homolog of the extracellular domain of c-MET, TEM1 or IGF-1R;
 - (b) providing host cells expressing

- (i) the human wt of the extracellular domain of the extracellular domain of c-MET (SEQ ID NO: 439), TEM1 (SEQ ID NO: 443) or IGF-1R (SEQ ID NO: 446) on the cell surface;
- (ii) a fusion protein comprising the human membrane proximal 640 amino acid residues identified in step (a) and the rodent amino acid residues >640 of c-MET, TEM1 or IGF-1R; and
- (iii) the rodent wt extracellular domain of c-MET, TEM1 or IGF-1R;
- (c) contacting the host cells according to step (b) with the bispecific single chain antibodies and effector T cells; and
- (d) identifying and isolating the bispecific single chain antibodies that mediate the lysis of host cells according to (b)(i) and (b)(ii) but not of host cells according to b(iii).

As described herein above, c-MET, TEM1 and IGF-1R are type I or type II integral membrane proteins with an extracellular portion of more than 640 amino acids. The consecutive sequence of the extracellular domain comprises continuous stretches of extracellular amino acids within the primary protein sequence and independently folded extracellular subdomain formed by a single continuous stretches.

Hepatocyte growth factor receptor MET (C-MET) is involved in the progression and spread of numerous human cancer types. The *MET* oncogene, encoding the receptor tyrosin kinase (RTK) for hepatocyte growth factor (HGF) and Scatter Factor (SF), controls genetic programs leading to cell growth, invasion, and protection from apoptosis. Deregulated activation of MET is critical not only for the acquisition of tumorigenic properties but also for the achievement of the invasive phenotype (Trusolino, L. & Comoglio, P. M. (2002) Nat. Rev. Cancer 2, 289–300). The role of *MET* in human tumors emerged from several experimental approaches and was unequivocally proven by the discovery of *MET*-activating mutations in inherited forms of carcinomas (Schmidt et al., Nat. Genet. 16 (1997), 68–73; Kim et al., J. Med. Genet. 40 (2003), e97). MET constitutive activation is frequent in sporadic cancers, and several studies have shown that the *MET* oncogene is overexpressed in tumors of specific histotypes or is activated through autocrine mechanisms (for a list see <http://www.vai.org/met/>). Besides, the *MET* gene is amplified in hematogenous metastases of colorectal carcinomas (Di Renzo et al., Clin. Cancer Res. 1 (1995), 147–154). The Scatter Factor (SF) secreted in culture by fibroblasts, that have the ability to induce intercellular dissociation of epithelial cells, and the Hepatocyte

Growth Factor (HGF), a potent mitogen for hepatocytes in culture derived from platelets or from blood of patients with acute liver failure, independently identified as Met ligands turned out to be the same molecule. Met and SF/HGF are widely expressed in a variety of tissues. The expression of Met (the receptor) is normally confined to cells of epithelial origin, while the expression of SF/HGF (the ligand) is restricted to cells of mesenchymal origin. Met is a transmembrane protein produced as a single-chain precursor. The precursor is proteolytically cleaved at a furin site to produce a highly glycosylated and entirely extracellular α -subunit of 50 kd and a β -subunit of 145 kd with a large extracellular region (involved in binding the ligand), a membrane spanning segment, and an intracellular region (containing the catalytic activity) (Giordano (1989) 339: 155-156). The α and β chains are disulphide linked. The extracellular portion of Met contains a region of homology to semaphorins (Sema domain, which includes the full α chain and the N-terminal part of the β chain of Met), a cysteine-rich Met Related Sequence (MRS) followed by glycineproline- rich (G-P) repeats, and four Immunoglobuline-like structures (Birchmeier et al., Nature Rev. 4 (2003), 915-25). The intracellular region of Met contains three regions: (1) a juxtamembrane segment that contains: (a) a serine residue (Ser 985) that, when phosphorylated by protein kinase C or by Ca^{2+} calmodulin-dependent kinases downregulates the receptor kinase activity Gandino et al., J. Biol. Chem. 269 (1994), 1815-20; and (b) a tyrosine (Tyr 1003) that binds the ubiquitin ligase Cbl responsible for Met polyubiquitination, endocytosis and degradation (Peschard et al., Mol. Cell 8 (2001), 995-1004); (2) the tyrosine kinase domain that, upon receptor activation, undergoes transphosphorylation on Tyr1234 and Tyr1235; (3) the C-terminal region, which comprises two crucial tyrosines (Tyr1349 and Tyr1356) inserted in a degenerate motif that represents a multisubstrate docking site capable of recruiting several downstream adaptors containing Src homology-2 (SH2) domains Met receptor, as most Receptor Tyrosine Kinases (RTKs) use different tyrosines to bind specific signaling molecules. The two tyrosines of the docking sites have been demonstrated to be necessary and sufficient for the signal transduction both in vitro and in vivo (Maina et al., Cell 87 (1996), 531-542; Ponzetto et al., Cell 77 (1994), 261-71).

A further example for a molecule having a large extracellular domain is the tumor endothelial marker (TEM) Endosialin (= TEM1). TEMs are overexpressed during tumor angiogenesis (St. Croix et al., Science 289 (2000), 1197-1202). Despite the

fact that their functions have not been characterized in detail so far, it is well established that they are strongly expressed on vascular endothelial cells in developing embryos and tumors studies (Carson-Walter et al., Cancer Res. 61: 6649-6655, 2001). Accordingly, Endosialin, a 165-kDa type I transmembrane protein, is expressed on the cell surface of tumor blood vessel endothelium in a broad range of human cancers but not detected in blood vessels or other cell types in many normal tissues. It is a C-type lectin-like molecule of 757 amino acids composed of a signal leader peptide, five globular extracellular domains (including a C-type lectin domain, one domain with similarity to the Sushi/ccp/scr pattern, and three EGF repeats), followed by a mucin like region, a transmembrane segment, and a short cytoplasmic tail (Christian et al., J. Biol. Chem. 276: 7408-7414, 2001). The Endosialin core protein carries abundantly sialylated, O-linked oligosaccharides and is sensitive to O-sialoglycoprotein endopeptidase, placing it in the group of sialomucin-like molecules. The N-terminal 360 amino acids of Endosialin show homology to thrombomodulin, a receptor involved in regulating blood coagulation, and to complement receptor C1qRp. This structural relationship indicates a function for Endosialin as a tumor endothelial receptor. Although Endosialin mRNA is ubiquitously expressed on endothelial cells in normal human and murine somatic tissues, Endosialin protein is largely restricted to the corpus luteum and highly angiogenic tissues such as the granular tissue of healing wounds or tumors (Opavsky et al., J. Biol. Chem. 276 (2001), 38795-38807; Rettig et al., PNAS 89 (1992), 10832-36). Endosialin protein expression is upregulated on tumor endothelial cells of carcinomas (breast, kidney, lung, colorectal, colon, pancreas mesothelioma), sarcomas, and neuroectodermal tumors (melanoma, glioma, neuroblastoma) (Rettig et al., loc. cit.). In addition, Endosialin is expressed at a low level on a subset of tumor stroma fibroblasts (Brady et al., J. Neuropathol. Exp. Neurol. 63 (2004), 1274-83; Opavsky et al., loc. cit.). Because of its restricted normal tissue distribution and abundant expression on tumor endothelial cells of many different types of solid tumors, Endosialin has been discussed as a target for antibody-based antiangiogenic treatment strategies of cancer. However, so far, there are no effective therapeutic approaches using Endosialin as a tumor endothelial target.

A still further example for a large antigen is the insulin-like growth factor I receptor (IGF-IR or IGF-1R). IGF-IR is a receptor with tyrosine kinase activity having 70% homology with the insulin receptor IR. IGF-IR is a glycoprotein of molecular weight

approximately 350,000. It is a hetero-tetrameric receptor of which each half-linked by disulfide bridges-is composed of an extracellular α -subunit and of a transmembrane [beta]-subunit. IGF-IR binds IGF I and IGF II with a very high affinity but is equally capable of binding to insulin with an affinity 100 to 1000 times less. Conversely, the IR binds insulin with a very high affinity although the IGFs only bind to the insulin receptor with a 100 times lower affinity. The tyrosine kinase domain of IGF-IR and of IR has a very high sequence homology although the zones of weaker homology respectively concern the cysteine-rich region situated on the alpha-subunit and the C-terminal part of the [beta]-subunit. The sequence differences observed in the α -subunit are situated in the binding zone of the ligands and are therefore at the origin of the relative affinities of IGF-IR and of IR for the IGFs and insulin respectively. The differences in the C-terminal part of the [beta]-subunit result in a divergence in the signalling pathways of the two receptors; IGF-IR mediating mitogenic, differentiation and antiapoptosis effects, while the activation of the IR principally involves effects at the level of the metabolic pathways (Baserga et al., *Biochim. Biophys. Acta*, 1332: F105-126, 1997; Baserga R., *Exp. Cell. Res.*, 253:1-6, 1999). The cytoplasmic tyrosine kinase proteins are activated by the binding of the ligand to the extracellular domain of the receptor. The activation of the kinases in its turn involves the stimulation of different intra-cellular substrates, including IRS-1, IRS-2, Shc and Grb 10 (Peruzzi F. et al., *J. Cancer Res. Clin. Oncol.*, 125:166-173, 1999). The two major substrates of IGF-IR are IRS and Shc which mediate, by the activation of numerous effectors downstream, the majority of the growth and differentiation effects connected with the attachment of the IGFs to this receptor. The availability of substrates can consequently dictate the final biological effect connected with the activation of the IGF-IR. When IRS-1 predominates, the cells tend to proliferate and to transform. When Shc dominates, the cells tend to differentiate (Valentinis B. et al.; *J. Biol. Chem.* 274:12423-12430, 1999). It seems that the route principally involved for the effects of protection against apoptosis is the phosphatidyl-inositol 3-kinases (PI 3-kinases) route (Prisco M. et al., *Horm. Metab. Res.*, 31:80-89, 1999; Peruzzi F. et al., *J. Cancer Res. Clin. Oncol.*, 125:166-173, 1999). The role of the IGF system in carcinogenesis has become the subject of intensive research in the last ten years. This interest followed the discovery of the fact that in addition to its mitogenic and antiapoptosis properties, IGF-IR seems to be required for the establishment and the maintenance of a transformed phenotype. In fact, it has been well established that an

overexpression or a constitutive activation of IGF-IR leads, in a great variety of cells, to a growth of the cells independent of the support in media devoid of fetal calf serum, and to the formation of tumors in nude mice. This in itself is not a unique property since a great variety of products of overexpressed genes can transform

5 cells, including a good number of receptors of growth factors. However, the crucial discovery which has clearly demonstrated the major role played by, IGF-IR in the transformation has been the demonstration that the R-cells, in which the gene coding for IGF-IR has been inactivated, are totally refractory to transformation by different agents which are usually capable of transforming the cells, such as the E5 protein of

10 bovine papilloma virus, an overexpression of EGFR or of PDGFR, the T antigen of SV 40, activated ras or the combination of these two last factors (Sell C. et al., Proc. Natl. Acad. Sci., USA, 90: 11217-11221, 1993; Sell C. et al., Mol. Cell. Biol., 14:3604-3612, 1994; Morrione A. J., Virol., 69:5300-5303, 1995; Coppola D. et al., Mol. Cell. Biol., 14:458a-4595, 1994; DeAngelis T et al., J. Cell. Physiol., 164:214-221, 1995).

15 IGF-IR is expressed in a great variety of tumors and of tumor lines and the IGFs amplify the tumor growth via their attachment to IGF-IR. Other arguments in favor of the role of IGF-IR in carcinogenesis come from studies using murine monoclonal antibodies directed against the receptor or using negative dominants of IGF-IR. In effect, murine monoclonal antibodies directed against IGF-IR inhibit the proliferation

20 of numerous cell lines in culture and the growth of tumor cells in vivo (Arteaga C. et al., Cancer Res., 49:6237-6241, 1989 Li et al., Biochem. Biophys. Res. Com., 196:92-98, 1993; Zia F et al., J. Cell. Biol., 24:269-275, 1996; Scotlandi K et al., Cancer Res., 58:4127-4131, 1998). It has likewise been shown in the works of Jiang et al. (Oncogene, 18:6071-6077, 1999) that a negative dominant of IGF-IR is capable

25 of inhibiting tumor proliferation.

The term "cell surface antigen" as used herein denotes a molecule, which is displayed on the surface of a cell. In most cases, this molecule will be located in or on the plasma membrane of the cell such that at least part of this molecule remains

30 accessible from outside the cell in tertiary form. A non-limiting example of a cell surface molecule, which is located in the plasma membrane is a transmembrane protein comprising, in its tertiary conformation, regions of hydrophilicity and hydrophobicity. Here, at least one hydrophobic region allows the cell surface molecule to be embedded, or inserted in the hydrophobic plasma membrane of the

cell while the hydrophilic regions extend on either side of the plasma membrane into the cytoplasm and extracellular space, respectively. Non-limiting examples of cell surface molecules which are located on the plasma membrane are proteins which have been modified at a cysteine residue to bear a palmitoyl group, proteins modified at a C-terminal cysteine residue to bear a farnesyl group or proteins which have been modified at the C-terminus to bear a glycosyl phosphatidyl inositol ("GPI") anchor. These groups allow covalent attachment of proteins to the outer surface of the plasma membrane, where they remain accessible for recognition by extracellular molecules such as antibodies. Examples of cell surface antigens are CD3 (in particular CD3 ϵ), PSMA, FAP α , c-MET, endosialin and IGF-IR. As described herein above, PSMA, FAP α , c-MET, endosialin and IGF-IR are cell surface antigens which are targets for therapy of cancer, including, but not limited to solid tumors.

In light of this, the target antigens PSMA, FAP α , c-MET, endosialin and IGF-IR can also be characterized as tumor antigens. The term "tumor antigen" as used herein may be understood as those antigens that are presented on tumor cells. These antigens can be presented on the cell surface with an extracellular part, which is often combined with a transmembrane and cytoplasmic part of the molecule. These antigens can sometimes be presented only by tumor cells and never by the normal ones. Tumor antigens can be exclusively expressed on tumor cells or might represent a tumor specific mutation compared to normal cells. In this case, they are called tumor-specific antigens. More common are antigens that are presented by tumor cells and normal cells, and they are called tumor-associated antigens. These tumor-associated antigens can be overexpressed compared to normal cells or are accessible for antibody binding in tumor cells due to the less compact structure of the tumor tissue compared to normal tissue.

In accordance with the present invention an independently folded protein domain is defined as a discrete portion of a protein formed by a single continuous stretch of amino acids within the primary protein sequence, e.g. known from its crystal structure, to take the "correct conformation" without requiring support by other portions of the protein or predicted to do so by comparison with hidden Markov models in libraries of described sequence domains, such as PFAM (Bateman (2000) Nucleic Acids Res. 28: 263-266) and SMART (Schultz (2000) Nucleic Acids Res. 28:

231-234), sequence similarity searches in data bases with the BLAST and PSI-BLAST tools (Altschul (1997) Nucleic Acids Res. 25: 3389-3402) that rely on the concept of a common evolutionary ancestor among sequentially homologous sequences or any other state-of-the-art domain prediction method. Independently
5 folded domains of the same protein chain are often joined by a flexible segment of amino acids, with each half of the flexible segment counting to its adjacent independently folded protein domain. Independently folded domains of the same protein may be connected in a precursor molecule by a protease cleavage site and after proteolytical processing may lie on two different connected protein chains in the
10 mature molecule. Independently folded protein domains may comprise functionally and/or structurally defined subdomains which do not take their correct conformation without requiring support by other portions of the protein because they are formed by discontinuous stretches of extracellular amino acids within the primary protein sequence or kept in their correct conformation by adjacent or other portions of the
15 protein.

Ther term "method for the selection", respectively the term "selecting" denotes in the context of the present invention the identification and isolation of one or more bispecific single chain antibodies from a population of candidate antibodies. In
20 particular, the candidate antibodies are tested in separate settings for the binding and the mediation of cytotoxicity for each of the three different host cell populations. Populations of bispecific single chain antibodies to be tested and methods for the generation of such populations are described in the appended examples. Since the method of the invention allows for the isolation of one ore more bispecific single
25 chain antibodies the method is also understood as a method for the production of bispecific single chain antibodies of the invention. Of course, such method for the production involves the production of the population of bispecific single chain antibodies, from which the one or more, which bind to the membrane proximal epitopes, are isolated.

30 As used herein, a "bispecific single chain antibody" denotes a single polypeptide chain comprising two binding domains. Each binding domain comprises one variable region from an antibody heavy chain ("VH region"), wherein the VH region of the first binding domain specifically binds to the CD3 molecule, and the VH region of the second binding domain specifically binds to the extracellular domain of a membrane

protein on a target cell, e.g. to PSMA, FAP α , c-MET, endosialin/TEM1 or IGF-1R. The two binding domains are optionally linked to one another by a short polypeptide spacer. A non-limiting example for a polypeptide spacer is Gly-Gly-Gly-Gly-Ser (G-G-G-G-S) and repeats thereof. Each binding domain may additionally comprise one
5 variable region from an antibody light chain ("VL region"), the VH region and VL region within each of the first and second binding domains being linked to one another via a polypeptide linker, for example of the type disclosed and claimed in EP 623679 B1, but in any case long enough to allow the VH region and VL region of the first binding domain and the VH region and VL region of the second binding domain
10 to pair with one another such that, together, they are able to specifically bind to the respective first and second binding domains.

The term "protein" is well known in the art and describes biological compounds. Proteins comprise one or more amino acid chains (polypeptides), whereby the amino
15 acids are bound among one another via a peptide bond. The term "polypeptide" as used herein describes a group of molecules, which consists of more than 30 amino acids. In accordance with the invention, the group of polypeptides comprises "proteins" as long as the proteins consist of a single polypeptide chain. Also in line with the definition the term "polypeptide" describes fragments of proteins as long as
20 these fragments consist of more than 30 amino acids. Polypeptides may further form multimers such as dimers, trimers and higher oligomers, i.e. consisting of more than one polypeptide molecule. Polypeptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding higher order structures of such multimers are, consequently, termed homo- or heterodimers, homo- or heterotrimers
25 etc. An example for a hereteromultimer is an antibody molecule, which, in its naturally occurring form, consists of two identical light polypeptide chains and two identical heavy polypeptide chains. The terms "polypeptide" and "protein" also refer to naturally modified polypeptides/proteins wherein the modification is effected e.g. by post-translational modifications like glycosylation, acetylation, phosphorylation and
30 the like. Such modifications are well known in the art.

The term "binding domain" characterizes in connection with the present invention a domain of a polypeptide which specifically binds to/interacts with a given target structure/antigen/epitope. Thus, the binding domain is an "antigen-interaction-site".

The term “antigen-interaction-site” defines, in accordance with the present invention, a motif of a polypeptide, which is able to specifically interact with a specific antigen or a specific group of antigens, e.g. the identical antigen in different species. Said binding/interaction is also understood to define a “specific recognition”. The term “specifically recognizing” means in accordance with this invention that the antibody molecule is capable of specifically interacting with and/or binding to at least two, preferably at least three, more preferably at least four amino acids of an antigen, e.g. the human CD3 antigen and the target antigens as defined herein. Such binding may be exemplified by the specificity of a “lock-and-key-principle”. Thus, specific motifs in the amino acid sequence of the binding domain and the antigen bind to each other as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structure. The specific interaction of the antigen-interaction-site with its specific antigen may result as well in a simple binding of said site to the antigen. Moreover, the specific interaction of the binding domain/antigen-interaction-site with its specific antigen may alternatively result in the initiation of a signal, e.g. due to the induction of a change of the conformation of the antigen, an oligomerization of the antigen, etc.

The term “antibody” comprises derivatives or functional fragments thereof which still retain the binding specificity. Techniques for the production of antibodies are well known in the art and described, e.g. in Harlow and Lane “Antibodies, A Laboratory Manual”, Cold Spring Harbor Laboratory Press, 1988 and Harlow and Lane “Using Antibodies: A Laboratory Manual” Cold Spring Harbor Laboratory Press, 1999. The term “antibody” also comprises immunoglobulins (Ig’s) of different classes (i.e. IgA, IgG, IgM, IgD and IgE) and subclasses (such as IgG1, IgG2 etc.).

The definition of the term “antibody” also includes embodiments such as chimeric, single chain and humanized antibodies, as well as antibody fragments, like, inter alia, Fab fragments. Antibody fragments or derivatives further comprise F(ab')₂, Fv, scFv fragments or single domain antibodies, single variable domain antibodies or immunoglobulin single variable domain comprising merely one variable domain, which might be VH or VL, that specifically bind to an antigen or epitope independently of other V regions or domains; see, for example, Harlow and Lane (1988) and (1999), loc. cit. Such immunoglobulin single variable domain encompasses not only an isolated antibody single variable domain polypeptide, but

also larger polypeptides that comprise one or more monomers of an antibody single variable domain polypeptide sequence.

Various procedures are known in the art and may be used for the production of such antibodies and/or fragments. Thus, the (antibody) derivatives can also be produced by peptidomimetics. Further, techniques described for the production of single chain antibodies (see, inter alia, US Patent 4,946,778) can be adapted to produce single chain antibodies specific for elected polypeptide(s). Also, transgenic animals may be used to express humanized or human antibodies specific for polypeptides and fusion proteins of this invention. For the preparation of monoclonal antibodies, any technique, providing antibodies produced by continuous cell line cultures can be used. Examples for such techniques include the hybridoma technique (Köhler and Milstein Nature 256 (1975), 495-497), the trioma technique, the human B-cell hybridoma technique (Kozbor, Immunology Today 4 (1983), 72) and the EBV-hybridoma technique to produce human monoclonal antibodies (Cole et al., Monoclonal Antibodies and Cancer Therapy, Alan R. Liss, Inc. (1985), 77-96). Surface plasmon resonance as employed in the BIAcore system can be used to increase the efficiency of phage antibodies which bind to an epitope of a target polypeptide, such as CD3 (epsilon), PSMA or FAP α , c-MET, TEM1 or IGF-1R (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmberg, J. Immunol. Methods 183 (1995), 7-13). It is also envisaged in the context of this invention that the term "antibody" comprises antibody constructs, which may be expressed in a host as described herein below, e.g. antibody constructs which may be transfected and/or transduced via, inter alia, viruses or plasmid vectors.

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The term "specific interaction" as used in accordance with the present invention means that the binding domain does not or does not significantly cross-react with polypeptides which have similar structure as those bound by the binding domain, and which might be expressed by the same cells as the polypeptide of interest. Cross-reactivity of a panel of binding domains under investigation may be tested, for example, by assessing binding of said panel of binding domains under conventional conditions (see, e.g., Harlow and Lane, Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1988 and Using Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory Press, 1999). Examples for the specific interaction of

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a binding domain with a specific antigen comprise the specificity of a ligand for its receptor. Said definition particularly comprises the interaction of ligands, which induce a signal upon binding to its specific receptor. Examples for said interaction, which is also particularly comprised by said definition, is the interaction of an
5 antigenic determinant (epitope) with the binding domain (antigenic binding site) of an antibody.

According to a preferred embodiment of the method of the invention the first binding domain binds to CD3 epsilon (CD3 ϵ) of human and non-chimpanzee primate. In this
10 context it is particularly preferred that the first binding domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain binds to an epitope, which is part of an amino acid sequence comprised in the group consisting of SEQ ID NOs. 2, 4, 6, and 8.

As used herein, "human" and "man" refers to the species *Homo sapiens*. As far as
15 the medical uses of the constructs described herein are concerned, human patients are to be treated with the same molecule.

The term "human" antibody as used herein is to be understood as meaning that the bispecific single chain antibody as defined herein, comprises (an) amino acid sequence(s) contained in the human germline antibody repertoire. For the purposes
20 of definition herein, said bispecific single chain antibody may therefore be considered human if it consists of such (a) human germline amino acid sequence(s), i.e. if the amino acid sequence(s) of the bispecific single chain antibody in question is (are) identical to (an) expressed human germline amino acid sequence(s). A bispecific single chain antibody as defined herein may also be regarded as human if it consists
25 of (a) sequence(s) that deviate(s) from its (their) closest human germline sequence(s) by no more than would be expected due to the imprint of somatic hypermutation. Additionally, the antibodies of many non-human mammals, for example rodents such as mice and rats, comprise VH CDR3 amino acid sequences which one may expect to exist in the expressed human antibody repertoire as well.
30 Any such sequence(s) of human or non-human origin which may be expected to exist in the expressed human repertoire would also be considered "human" for the purposes of the present invention.

Though T cell-engaging bispecific single chain antibodies described in the art have

great therapeutic potential for the treatment of malignant diseases, most of these bispecific molecules are limited in that they are species specific and recognize only human antigen, and - due to genetic similarity - likely the chimpanzee counterpart. The advantage of the preferred embodiment of the invention is the provision of a
5 bispecific single chain antibody comprising a binding domain exhibiting cross-species specificity to human and non-chimpanzee primate of the CD3 epsilon chain.

Herein described examples for preferred first binding domains bind to an N-terminal 1-27 amino acid residue polypeptide fragment of the extracellular domain of CD3 epsilon. This 1-27 amino acid residue polypeptide fragment was surprisingly
10 identified which - in contrast to all other known epitopes of CD3 epsilon described in the art - maintains its three-dimensional structural integrity when taken out of its native environment in the CD3 complex (and optionally fused to a heterologous amino acid sequence such as EpCAM or an immunoglobulin Fc part).

The present invention, therefore, provides for a bispecific single chain antibody
15 molecule comprising a first binding domain capable of binding to an epitope of an N-terminal 1-27 amino acid residue polypeptide fragment of the extracellular domain of CD3 epsilon (which CD3 epsilon is, for example, taken out of its native environment and/or comprised by (presented on the surface of) a T-cell) of human and at least one non-chimpanzee primate CD3 epsilon chain, wherein the epitope is part of an
20 amino acid sequence comprised in the group consisting of SEQ ID NOs. 2, 4, 6, and 8; and a second binding domain capable of binding to prostate-specific membrane antigen (PSMA). Preferred non-chimpanzee primates are mentioned herein elsewhere. At least one (or a selection thereof or all) primate(s) selected from *Callithrix jacchus*; *Saguinus oedipus*, *Saimiri sciureus*, and *Macaca fascicularis*
25 (either SEQ ID 863 or 864 or both), is (are) particularly preferred. *Macaca mulatta*, also known as Rhesus Monkey is also envisaged as another preferred primate. It is thus envisaged that antibodies of the invention bind to (are capable of binding to) the context independent epitope of an N-terminal 1-27 amino acid residue polypeptide fragment of the extracellular domain of CD3 epsilon of human and *Callithrix jacchus*,
30 *Saguinus oedipus*, *Saimiri sciureus*, and *Macaca fascicularis* (either SEQ ID 863 or 864 or both), and optionally also to *Macaca mulatta*. A bispecific single chain antibody molecule comprising a first binding domain as defined herein can be obtained (is obtainable by) or can be manufactured in accordance with the protocol set out in the appended Examples (in particular Example 2). To this end, it is

envisaged to (a) immunize mice with an N-terminal 1-27 amino acid residue polypeptide fragment of the extracellular domain of CD3 epsilon of human and/or Saimiri sciureus; (b) generation of an immune murine antibody scFv library; (c) identification of CD3 epsilon specific binders by testing the capability to bind to at least SEQ ID NOs. 2, 4, 6, and 8.

The context-independence of the CD3 epitope provided herein corresponds to the first 27 N-terminal amino acids of CD3 epsilon or functional fragments of this 27 amino acid stretch. The phrase "context-independent," as used herein in relation to the CD3 epitope means that binding of the herein described inventive binding molecules/antibody molecules does not lead to a change or modification of the conformation, sequence, or structure surrounding the antigenic determinant or epitope. In contrast, the CD3 epitope recognized by a conventional CD3 binding molecule (e.g. as disclosed in WO 99/54440 or WO 04/106380) is localized on the CD3 epsilon chain C-terminally to the N-terminal 1-27 amino acids of the context-independent epitope, where it only takes the correct conformation if it is embedded within the rest of the epsilon chain and held in the right sterical position by heterodimerization of the epsilon chain with either the CD3 gamma or delta chain.

Anti-CD3 binding domains as part of bispecific single chain molecules as provided herein and generated (and directed) against a context-independent CD3 epitope provide for a surprising clinical improvement with regard to T cell redistribution and, thus, a more favourable safety profile. Without being bound by theory, since the CD3 epitope is context-independent, forming an autonomous selfsufficient subdomain without much influence on the rest of the CD3 complex, the CD3 binding domain of the bispecific single chain molecules provided herein induces less allosteric changes in CD3 conformation than the conventional CD3 binding molecules (like molecules provided in WO 99/54440 or WO 04/106380), which recognize context-dependent CD3 epitopes.

The context-independence of the CD3 epitope which is recognized by the CD3 binding domain of the bispecific single chain antibodies of the invention, respectively isolated by the method of the invention, is associated with less or no T cell redistribution (T cell redistribution equates with an initial episode of drop and subsequent recovery of absolute T cell counts) during the starting phase of treatment with said bispecific single chain antibody. This results in a better safety profile of the bispecific single chain antibodies of the invention compared to conventional CD3

binding molecules known in the art, which recognize context-dependent CD3 epitopes. Particularly, because T cell redistribution during the starting phase of treatment with CD3 binding molecules is a major risk factor for adverse events, like CNS adverse events, the bispecific single chain antibodies of the invention by recognizing a context-independent rather than a context-dependent CD3 epitope has a substantial safety advantage over the CD3 binding molecules known in the art. Patients with such CNS adverse events related to T cell redistribution during the starting phase of treatment with conventional CD3 binding molecules usually suffer from confusion and disorientation, in some cases also from urinary incontinence. Confusion is a change in mental status in which the patient is not able to think with his or her usual level of clarity. The patient usually has difficulties to concentrate and thinking is not only blurred and unclear but often significantly slowed down. Patients with CNS adverse events related to T cell redistribution during the starting phase of treatment with conventional CD3 binding molecules may also suffer from loss of memory. Frequently, the confusion leads to the loss of ability to recognize people, places, time or the date. Feelings of disorientation are common in confusion, and the decision-making ability is impaired. CNS adverse events related to T cell redistribution during the starting phase of treatment with conventional CD3 binding molecules may further comprise blurred speech and/or word finding difficulties. This disorder may impair both, the expression and understanding of language as well as reading and writing. Besides urinary incontinence, vertigo and dizziness may also accompany CNS adverse events related to T cell redistribution during the starting phase of treatment with conventional CD3 binding molecules in some patients. The maintenance of the three-dimensional structure within the mentioned 27 amino acid N-terminal polypeptide fragment of CD3 epsilon can be used for the generation of, preferably human, binding domains which are capable of binding to the N-terminal CD3 epsilon polypeptide fragment *in vitro* and to the native (CD3 epsilon subunit of the) CD3 complex on T cells *in vivo* with the same binding affinity. These data strongly indicate that the N-terminal fragment as described herein forms a tertiary conformation, which is similar to its structure normally existing *in vivo*. A very sensitive test for the importance of the structural integrity of the amino acids 1-27 of the N-terminal polypeptide fragment of CD3 epsilon was performed. Individual amino acids of amino acids 1-27 of the N-terminal polypeptide fragment of CD3 epsilon

were changed to alanine (alanine scanning) to test the sensitivity of the amino acids 1-27 of the N-terminal polypeptide fragment of CD3 epsilon for minor disruptions.

Unexpectedly, it has been found that the thus isolated, preferably human, bispecific
5 single chain antibody of the invention not only recognizes the human N-terminal
fragment of CD3 epsilon, but also the corresponding homologous fragments of CD3
epsilon of various primates, including New-World Monkeys (Marmoset, *Callithrix*
jacchus; *Saguinus oedipus*; *Saimiri sciureus*) and Old-World Monkeys (*Macaca*
fascicularis, also known as Cynomolgus Monkey; or *Macaca mulatta*, also known as
10 Rhesus Monkey). Thus, multi-primate specificity of the bispecific single chain
antibodies of the invention can be detected. The multi-primate specificity of the binding
domains of the invention is defined herein as cross-species specificity.

The amino acid sequence of the aforementioned N-terminal fragments of CD3 epsilon
are depicted in SEQ ID No. 2 (human), SEQ ID No. 4 (*Callithrix jacchus*); SEQ ID No.
15 6 (*Saguinus oedipus*); SEQ ID No. 8 (*Saimiri sciureus*); SEQ ID No. **863**
QDGNEEMGSITQTPYQVSISGTTILTC or SEQ ID No. **864**
QDGNEEMGSITQTPYQVSISGTTVILT (*Macaca fascicularis*, also known as
Cynomolgus Monkey), and SEQ ID No. **865** *QDGNEEMGSITQTPYHVSISGTTVILT*
(*Macaca mulatta*, also known as Rhesus Monkey).

20 The term "cross-species specificity" or "interspecies specificity" as used herein
means binding of a binding domain described herein to the same target molecule in
humans and non-chimpanzee primates. Thus, "cross-species specificity" or
"interspecies specificity" is to be understood as an interspecies reactivity to the same
25 molecule "X" expressed in different species, but not to a molecule other than "X".
Cross-species specificity of a monoclonal antibody recognizing e.g. human CD3
epsilon, to a non-chimpanzee primate CD3 epsilon, e.g. macaque CD3 epsilon, can
be determined, for instance, by FACS analysis. The FACS analysis is carried out in a
way that the respective monoclonal antibody is tested for binding to human and non-
30 chimpanzee primate cells, e.g. macaque cells, expressing said human and non-
chimpanzee primate CD3 epsilon antigens, respectively. An appropriate assay is
shown in the following examples. The above-mentioned subject matter applies
mutatis mutandis for the target antigens PSMA, FAP α , endosialin (TEM1), c-MET and
IGF-1R: Cross-species specificity of a monoclonal antibody recognizing e.g. human

PSMA, to a non-chimpanzee primate PSMA, e.g. macaque PSMA, can be determined, for instance, by FACS analysis. The FACS analysis is carried out in a way that the respective monoclonal antibody is tested for binding to human and non-chimpanzee primate cells, e.g. macaque cells, expressing said human and non-chimpanzee primate PSMA antigens, respectively.

As used herein, CD3 epsilon denotes a molecule expressed as part of the T cell receptor and has the meaning as typically ascribed to it in the prior art. In human, it encompasses in individual or independently combined form all known CD3 subunits, for example CD3 epsilon, CD3 delta, CD3 gamma, CD3 zeta, CD3 alpha and CD3 beta. The non-chimpanzee primate, non-human CD3 antigens as referred to herein are, for example, *Macaca fascicularis* CD3 and *Macaca mulatta* CD3. In *Macaca fascicularis*, it encompasses CD3 epsilon FN-18 negative and CD3 epsilon FN-18 positive, CD3 gamma and CD3 delta. In *Macaca mulatta*, it encompasses CD3 epsilon, CD3 gamma and CD3 delta. Preferably, said CD3 as used herein is CD3 epsilon.

The human CD3 epsilon is indicated in GenBank Accession No. NM_000733 and comprises SEQ ID NO. 1. The human CD3 gamma is indicated in GenBank Accession NO. NM_000073. The human CD3 delta is indicated in GenBank Accession No. NM_000732.

The CD3 epsilon "FN-18 negative" of *Macaca fascicularis* (i.e. CD3 epsilon not recognized by monoclonal antibody FN-18 due to a polymorphism as set forth above) is indicated in GenBank Accession No. AB073994.

The CD3 epsilon "FN-18 positive" of *Macaca fascicularis* (i.e. CD3 epsilon recognized by monoclonal antibody FN-18) is indicated in GenBank Accession No. AB073993. The CD3 gamma of *Macaca fascicularis* is indicated in GenBank Accession No. AB073992. The CD3 delta of *Macaca fascicularis* is indicated in GenBank Accession No. AB073991.

The nucleic acid sequences and amino acid sequences of the respective CD3 epsilon, gamma and delta homologs of *Macaca mulatta* can be identified and isolated by recombinant techniques described in the art (Sambrook et al. Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, 3rd edition 2001). This applies mutatis mutandis to the CD3 epsilon, gamma and delta homologs of other non-chimpanzee primates as defined herein. The identification of the amino acid sequence of *Callithrix jacchus*, *Saimiri sciureus* und *Saguinus oedipus* is described in

the appended examples. The amino acid sequence of the extracellular domain of the CD3 epsilon of *Callithrix jacchus* is depicted in SEQ ID NO: 3, the one of *Saguinus oedipus* is depicted in SEQ ID NO: 5 and the one of *Saimiri sciureus* is depicted in SEQ ID NO: 7.

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In line with the above, the term “epitope” defines an antigenic determinant, which is specifically bound/identified by a binding domain as defined herein. The binding domain may specifically bind to/interact with conformational or continuous epitopes, which are unique for the target structure, e.g. the human and non-chimpanzee primate CD3 epsilon chain. A conformational or discontinuous epitope is characterized for polypeptide antigens by the presence of two or more discrete amino acid residues which are separated in the primary sequence, but come together on the surface of the molecule when the polypeptide folds into the native protein/antigen (Sela, (1969) Science 166, 1365 and Laver, (1990) Cell 61, 553-6). The two or more discrete amino acid residues contributing to the epitope are present on separate sections of one or more polypeptide chain(s). These residues come together on the surface of the molecule when the polypeptide chain(s) fold(s) into a three-dimensional structure to constitute the epitope. In contrast, a continuous or linear epitope consists of two or more discrete amino acid residues, which are present in a single linear segment of a polypeptide chain. Within the present invention, a “context-dependent” CD3 epitope refers to the conformation of said epitope. Such a context-dependent epitope, localized on the epsilon chain of CD3, can only develop its correct conformation if it is embedded within the rest of the epsilon chain and held in the right position by heterodimerization of the epsilon chain with either CD3 gamma or delta chain. In contrast, a context-independent CD3 epitope as provided herein refers to an N-terminal 1-27 amino acid residue polypeptide or a functional fragment thereof of CD3 epsilon. This N-terminal 1-27 amino acid residue polypeptide or a functional fragment thereof maintains its three-dimensional structural integrity and correct conformation when taken out of its native environment in the CD3 complex. The context-independency of the N-terminal 1-27 amino acid residue polypeptide or a functional fragment thereof, which is part of the extracellular domain of CD3 epsilon, represents, thus, an epitope which is completely different to the epitopes of CD3 epsilon described in connection with a method for the preparation of human binding molecules in WO 2004/106380. Said method used solely expressed

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recombinant CD3 epsilon. The conformation of this solely expressed recombinant CD3 epsilon differed from that adopted in its natural form, that is, the form in which the CD3 epsilon subunit of the TCR/CD3 complex exists as part of a noncovalent complex with either the CD3 delta or the CD3-gamma subunit of the TCR/CD3 complex. When such solely expressed recombinant CD3 epsilon protein is used as an antigen for selection of antibodies from an antibody library, antibodies specific for this antigen are identified from the library although such a library does not contain antibodies with specificity for self-antigens/autoantigens. This is due to the fact that solely expressed recombinant CD3 epsilon protein does not exist in vivo; it is not an autoantigen. Consequently, subpopulations of B cells expressing antibodies specific for this protein have not been depleted in vivo; an antibody library constructed from such B cells would contain genetic material for antibodies specific for solely expressed recombinant CD3 epsilon protein.

However, since the context-independent N-terminal 1-27 amino acid residue polypeptide or a functional fragment thereof is an epitope, which folds in its native form, binding domains in line with the present invention cannot be identified by methods based on the approach described in WO 2004/106380. Therefore, it could be verified in tests that binding molecules as disclosed in WO 2004/106380 are not capable of binding to the N-terminal 1-27 amino acid residues of the CD3 epsilon chain. Hence, conventional anti-CD3 binding molecules or anti-CD3 antibody molecules (e.g. as disclosed in WO 99/54440) bind CD3 epsilon chain at a position which is more C-terminally located than the context-independent N-terminal 1-27 amino acid residue polypeptide or a functional fragment provided herein. Prior art antibody molecules OKT3 and UCHT-1 have also a specificity for the epsilon-subunit of the TCR/CD3 complex between amino acid residues 35 to 85 and, accordingly, the epitope of these antibodies is also more C-terminally located. In addition, UCHT-1 binds to the CD3 epsilon chain in a region between amino acid residues 43 to 77 (Tunnacliffe, Int. Immunol. 1 (1989), 546-50; Kjer-Nielsen, PNAS 101, (2004), 7675-7680; Salmeron, J. Immunol. 147 (1991), 3047-52). Therefore, prior art anti-CD3 molecules do not bind to and are not directed against the herein defined context-independent N-terminal 1-27 amino acid residue epitope (or a functional fragment thereof). In particular, the state of the art fails to provide anti-CD3 molecules which specifically binds to the context-independent N-terminal 1-27 amino acid residue epitope and which are cross-species specific, i.e. bind to human and non-

chimpanzee primate CD3 epsilon.

As used herein, the term “humanized”, “humanization”, “human-like” or grammatically related variants thereof are used interchangeably to refer to a bispecific single chain antibody comprising in at least one of its binding domains at least one complementarity determining region (“CDR”) from a non-human antibody or fragment thereof. Humanization approaches are described for example in WO 91/09968 and US 6,407,213. As non-limiting examples, the term encompasses the case in which a variable region of at least one binding domain comprises a single CDR region, for example the third CDR region of the VH (CDRH3), from another non-human animal, for example a rodent, as well as the case in which a or both variable region/s comprise at each of their respective first, second and third CDRs the CDRs from said non-human animal. In the event that all CDRs of a binding domain of the bispecific single chain antibody have been replaced by their corresponding equivalents from, for example, a rodent, one typically speaks of “CDR-grafting”, and this term is to be understood as being encompassed by the term “humanized” or grammatically related variants thereof as used herein. The term “humanized” or grammatically related variants thereof also encompasses cases in which, in addition to replacement of one or more CDR regions within a VH and/or VL of the first and/or second binding domain further mutation/s (e.g. substitutions) of at least one single amino acid residue/s within the framework (“FR”) regions between the CDRs has/have been effected such that the amino acids at that/those positions correspond/s to the amino acid/s at that/those position/s in the animal from which the CDR regions used for replacement is/are derived. As is known in the art, such individual mutations are often made in the framework regions following CDR-grafting in order to restore the original binding affinity of the non-human antibody used as a CDR-donor for its target molecule. The term “humanized” may further encompass (an) amino acid substitution(s) in the CDR regions from a non-human animal to the amino acid(s) of a corresponding CDR region from a human antibody, in addition to the amino acid substitutions in the framework regions as described above.

As used herein, the term “homolog” or “homology” is to be understood as follows: Homology among proteins and DNA is often concluded on the basis of sequence similarity, especially in bioinformatics. For example, in general, if two or more genes have highly similar DNA sequences, it is likely that they are homologous. But

sequence similarity may arise from different ancestors: short sequences may be similar by chance, and sequences may be similar because both were selected to bind to a particular protein, such as a transcription factor. Such sequences are similar but not homologous. Sequence regions that are homologous are also called conserved. This is not to be confused with conservation in amino acid sequences in which the amino acid at a specific position has changed but the physio-chemical properties of the amino acid remain unchanged. Homologous sequences are of two types: orthologous and paralogous. Homologous sequences are orthologous if they were separated by a speciation event: when a species diverges into two separate species, the divergent copies of a single gene in the resulting species are said to be orthologous. Orthologs, or orthologous genes, are genes in different species that are similar to each other because they originated from a common ancestor. The strongest evidence that two similar genes are orthologous is the result of a phylogenetic analysis of the gene lineage. Genes that are found within one clade are orthologs, descended from a common ancestor. Orthologs often, but not always, have the same function. Orthologous sequences provide useful information in taxonomic classification studies of organisms. The pattern of genetic divergence can be used to trace the relatedness of organisms. Two organisms that are very closely related are likely to display very similar DNA sequences between two orthologs. Conversely, an organism that is further removed evolutionarily from another organism is likely to display a greater divergence in the sequence of the orthologs being studied. Homologous sequences are paralogous if they were separated by a gene duplication event: if a gene in an organism is duplicated to occupy two different positions in the same genome, then the two copies are paralogous. A set of sequences that are paralogous are called paralogs of each other. Paralogs typically have the same or similar function, but sometimes do not: due to lack of the original selective pressure upon one copy of the duplicated gene, this copy is free to mutate and acquire new functions. An example can be found in rodents such as rats and mice. Rodents have a pair of paralogous insulin genes, although it is unclear if any divergence in function has occurred. Paralogous genes often belong to the same species, but this is not necessary: for example, the hemoglobin gene of humans and the myoglobin gene of chimpanzees are paralogs. This is a common problem in bioinformatics: when genomes of different species have been sequenced and homologous genes have been found, one can not immediately conclude that these

genes have the same or similar function, as they could be paralogs whose function has diverged.

As used herein, a “non-chimpanzee primate” or “non-chimp primate” or grammatical variants thereof refers to any primate animal (i.e. not human) other than chimpanzee, i.e. other than an animal of belonging to the genus *Pan*, and including the species *Pan paniscus* and *Pan troglodytes*, also known as *Anthropopithecus troglodytes* or *Simia satyrus*. It will be understood, however, that it is possible that the antibodies of the invention can also bind with their first and/or second binding domain to the respective epitopes/fragments etc. of said chimpanzees. The intention is merely to avoid animal tests which are carried out with chimpanzees, if desired. It is thus also envisaged that in another embodiment the antibodies of the present invention also bind with their first and/or second binding domain to the respective epitopes of chimpanzees. A “primate”, “primate species”, “primates” or grammatical variants thereof denote/s an order of eutherian mammals divided into the two suborders of prosimians and anthropoids and comprising apes, monkeys and lemurs. Specifically, “primates” as used herein comprises the suborder *Strepsirrhini* (non-tarsier prosimians), including the infraorder *Lemuriformes* (itself including the superfamilies *Cheirogaleoidea* and *Lemuroidea*), the infraorder *Chiromyiformes* (itself including the family *Daubentoniidae*) and the infraorder *Lorisiformes* (itself including the families *Lorisidae* and *Galagidae*). “Primates” as used herein also comprises the suborder *Haplorrhini*, including the infraorder *Tarsiiformes* (itself including the family *Tarsiidae*), the infraorder *Simiiformes* (itself including the *Platyrrhini*, or New-World monkeys, and the *Catarrhini*, including the *Cercopithecoidea*, or Old-World Monkeys).

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The non-chimpanzee primate species may be understood within the meaning of the invention to be a lemur, a tarsier, a gibbon, a marmoset (belonging to New-World Monkeys of the family *Cebidae*) or an Old-World Monkey (belonging to the superfamily *Cercopithecoidea*).

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As used herein, an “Old-World Monkey” comprises any monkey falling in the superfamily *Cercopithecoidea*, itself subdivided into the families: the *Cercopithecinae*, which are mainly African but include the diverse genus of macaques which are Asian and North African; and the *Colobinae*, which include most

of the Asian genera but also the African colobus monkeys.

Specifically, within the subfamily *Cercopithecinae*, an advantageous non-chimpanzee primate may be from the Tribe *Cercopithecini*, within the genus *Allenopithecus* (Allen's Swamp Monkey, *Allenopithecus nigroviridis*); within the genus *Miopithecus* (Angolan Talapoin, *Miopithecus talapoin*; Gabon Talapoin, *Miopithecus ogouensis*); within the genus *Erythrocebus* (Patas Monkey, *Erythrocebus patas*); within the genus *Chlorocebus* (Green Monkey, *Chlorocebus sabaceus*; Grivet, *Chlorocebus aethiops*; Bale Mountains Vervet, *Chlorocebus djamdjamensis*; Tantalus Monkey, *Chlorocebus tantalus*; Vervet Monkey, *Chlorocebus pygerythrus*; Malbrouck, *Chlorocebus cynosuros*); or within the genus *Cercopithecus* (Dryas Monkey or Salongo Monkey, *Cercopithecus dryas*; Diana Monkey, *Cercopithecus diana*; Roloway Monkey, *Cercopithecus roloway*; Greater Spot-nosed Monkey, *Cercopithecus nictitans*; Blue Monkey, *Cercopithecus mitis*; Silver Monkey, *Cercopithecus doggetti*; Golden Monkey, *Cercopithecus kandti*; Sykes's Monkey, *Cercopithecus albogularis*; Mona Monkey, *Cercopithecus mona*; Campbell's Mona Monkey, *Cercopithecus campbelli*; Lowe's Mona Monkey, *Cercopithecus lowei*; Crested Mona Monkey, *Cercopithecus pogonias*; Wolf's Mona Monkey, *Cercopithecus wolffi*; Dent's Mona Monkey, *Cercopithecus denti*; Lesser Spot-nosed Monkey, *Cercopithecus petaurista*; White-throated Guenon, *Cercopithecus erythrogaster*; Sclater's Guenon, *Cercopithecus sclateri*; Red-eared Guenon, *Cercopithecus erythrotis*; Moustached Guenon, *Cercopithecus cephus*; Red-tailed Monkey, *Cercopithecus ascanius*; L'Hoest's Monkey, *Cercopithecus lhoesti*; Preuss's Monkey, *Cercopithecus preussi*; Sun-tailed Monkey, *Cercopithecus solatus*; Hamlyn's Monkey or Owl-faced Monkey, *Cercopithecus hamlyni*; De Brazza's Monkey, *Cercopithecus neglectus*).

Alternatively, an advantageous non-chimpanzee primate, also within the subfamily *Cercopithecinae* but within the Tribe *Papionini*, may be from within the genus *Macaca* (Barbary Macaque, *Macaca sylvanus*; Lion-tailed Macaque, *Macaca silenus*; Southern Pig-tailed Macaque or Beruk, *Macaca nemestrina*; Northern Pig-tailed Macaque, *Macaca leonina*; Pagai Island Macaque or Bokkoi, *Macaca pagensis*; Siberut Macaque, *Macaca siberu*; Moor Macaque, *Macaca maura*; Booted Macaque, *Macaca ochreata*; Tonkean Macaque, *Macaca tonkeana*; Heck's Macaque, *Macaca hecki*; Gorontalo Macaque, *Macaca nigriscens*; Celebes Crested Macaque or Black "Ape", *Macaca nigra*; Cynomolgus monkey or Crab-eating Macaque or Long-tailed Macaque or Kera, *Macaca fascicularis*; Stump-tailed Macaque or Bear Macaque,

Macaca arctoides; Rhesus Macaque, *Macaca mulatta*; Formosan Rock Macaque, *Macaca cyclopis*; Japanese Macaque, *Macaca fuscata*; Toque Macaque, *Macaca sinica*; Bonnet Macaque, *Macaca radiata*; Barbary Macaque, *Macaca sylvanus*; Assam Macaque, *Macaca assamensis*; Tibetan Macaque or Milne-Edwards' Macaque, *Macaca thibetana*; Arunachal Macaque or Munzala, *Macaca munzala*);
 5 within the genus *Lophocebus* (Gray-cheeked Mangabey, *Lophocebus albigena*; *Lophocebus albigena albigena*; *Lophocebus albigena osmani*; *Lophocebus albigena johnstoni*; Black Crested Mangabey, *Lophocebus aterrimus*; Opdenbosch's Mangabey, *Lophocebus opdenboschi*; Highland Mangabey, *Lophocebus kipunji*);
 10 within the genus *Papio* (Hamadryas Baboon, *Papio hamadryas*; Guinea Baboon, *Papio papio*; Olive Baboon, *Papio anubis*; Yellow Baboon, *Papio cynocephalus*; Chacma Baboon, *Papio ursinus*); within the genus *Theropithecus* (Gelada, *Theropithecus gelada*); within the genus *Cercocebus* (Sooty Mangabey, *Cercocebus atys*; *Cercocebus atys atys*; *Cercocebus atys lunulatus*; Collared Mangabey,
 15 *Cercocebus torquatus*; Agile Mangabey, *Cercocebus agilis*; Golden-bellied Mangabey, *Cercocebus chrysogaster*; Tana River Mangabey, *Cercocebus galeritus*; Sanje Mangabey, *Cercocebus sanjei*); or within the genus *Mandrillus* (Mandrill, *Mandrillus sphinx*; Drill, *Mandrillus leucophaeus*).

Most preferred is *Macaca fascicularis* (also known as Cynomolgus monkey and,
 20 therefore, in the Examples named "Cynomolgus") and *Macaca mulatta* (rhesus monkey, named "rhesus").

Within the subfamily *Colobinae*, an advantageous non-chimpanzee primate may be from the African group, within the genus *Colobus* (Black Colobus, *Colobus satanas*; Angola Colobus, *Colobus angolensis*; King Colobus, *Colobus polykomos*; Ursine
 25 Colobus, *Colobus vellerosus*; Mantled Guereza, *Colobus guereza*); within the genus *Piliocolobus* (Western Red Colobus, *Piliocolobus badius*; *Piliocolobus badius badius*; *Piliocolobus badius temminckii*; *Piliocolobus badius waldronae*; Pennant's Colobus, *Piliocolobus pennantii*; *Piliocolobus pennantii pennantii*; *Piliocolobus pennantii epieni*; *Piliocolobus pennantii bouvieri*; Preuss's Red Colobus, *Piliocolobus preussi*; Thollon's
 30 Red Colobus, *Piliocolobus tholloni*; Central African Red Colobus, *Piliocolobus foai*; *Piliocolobus foai foai*; *Piliocolobus foai ellioti*; *Piliocolobus foai oustaleti*; *Piliocolobus foai semlikiensis*; *Piliocolobus foai parmentierorum*; Ugandan Red Colobus, *Piliocolobus tephrosceles*; Uzungwa Red Colobus, *Piliocolobus gordonorum*; Zanzibar Red Colobus, *Piliocolobus kirkii*; Tana River Red Colobus, *Piliocolobus*

*rufomitratu*s); or within the genus *Procolobus* (Olive Colobus, *Procolobus verus*).

Within the subfamily *Colobinae*, an advantageous non-chimpanzee primate may alternatively be from the Langur (leaf monkey) group, within the genus *Semnopithecus* (Nepal Gray Langur, *Semnopithecus schistaceus*; Kashmir Gray Langur, *Semnopithecus ajax*; Tarai Gray Langur, *Semnopithecus hector*; Northern Plains Gray Langur, *Semnopithecus entellus*; Black-footed Gray Langur, *Semnopithecus hypoleucos*; Southern Plains Gray Langur, *Semnopithecus dussumieri*; Tufted Gray Langur, *Semnopithecus priam*); within the *T. vetulus* group or the genus *Trachypithecus* (Purple-faced Langur, *Trachypithecus vetulus*; Nilgiri Langur, *Trachypithecus johnii*); within the *T. cristatus* group of the genus *Trachypithecus* (Javan Lutung, *Trachypithecus auratus*; Silvery Leaf Monkey or Silvery Lutung, *Trachypithecus cristatus*; Indochinese Lutung, *Trachypithecus germaini*; Tenasserim Lutung, *Trachypithecus barbei*); within the *T. obscurus* group of the genus *Trachypithecus* (Dusky Leaf Monkey or Spectacled Leaf Monkey, *Trachypithecus obscurus*; Phayre's Leaf Monkey, *Trachypithecus phayrei*); within the *T. pileatus* group of the genus *Trachypithecus* (Capped Langur, *Trachypithecus pileatus*; Shortridge's Langur, *Trachypithecus shortridgei*; Gee's Golden Langur, *Trachypithecus geei*); within the *T. francoisi* group of the genus *Trachypithecus* (Francois' Langur, *Trachypithecus francoisi*; Hatinh Langur, *Trachypithecus hatinhensis*; White-headed Langur, *Trachypithecus poliocephalus*; Laotian Langur, *Trachypithecus laotum*; Delacour's Langur, *Trachypithecus delacouri*; Indochinese Black Langur, *Trachypithecus ebenus*); or within the genus *Presbytis* (Sumatran Surili, *Presbytis melalophos*; Banded Surili, *Presbytis femoralis*; Sarawak Surili, *Presbytis chrysomelas*; White-thighed Surili, *Presbytis siamensis*; White-fronted Surili, *Presbytis frontata*; Javan Surili, *Presbytis comata*; Thomas's Langur, *Presbytis thomasi*; Hose's Langur, *Presbytis hosei*; Maroon Leaf Monkey, *Presbytis rubicunda*; Mentawai Langur or Joja, *Presbytis potenziani*; Natuna Island Surili, *Presbytis natunae*).

Within the subfamily *Colobinae*, an advantageous non-chimpanzee primate may alternatively be from the Odd-Nosed group, within the genus *Pygathrix* (Red-shanked Douc, *Pygathrix nemaeus*; Black-shanked Douc, *Pygathrix nigripes*; Gray-shanked Douc, *Pygathrix cinerea*); within the genus *Rhinopithecus* (Golden Snub-nosed Monkey, *Rhinopithecus roxellana*; Black Snub-nosed Monkey, *Rhinopithecus bieti*; Gray Snub-nosed Monkey, *Rhinopithecus brelichi*; Tonkin Snub-nosed Langur,

Rhinopithecus avunculus); within the genus *Nasalis* (Proboscis Monkey, *Nasalis larvatus*); or within the genus *Simias* (Pig-tailed Langur, *Simias concolor*).

As used herein, the term “marmoset” denotes any New-World Monkeys of the genus *Callithrix*, for example belonging to the Atlantic marmosets of subgenus *Callithrix* (sic!) (Common Marmoset, *Callithrix (Callithrix) jacchus*; Black-tufted Marmoset, *Callithrix (Callithrix) penicillata*; Wied's Marmoset, *Callithrix (Callithrix) kuhlii*; White-headed Marmoset, *Callithrix (Callithrix) geoffroyi*; Buffy-headed Marmoset, *Callithrix (Callithrix) flaviceps*; Buffy-tufted Marmoset, *Callithrix (Callithrix) aurita*); belonging to the Amazonian marmosets of subgenus *Mico* (Rio Acari Marmoset, *Callithrix (Mico) acariensis*; Manicore Marmoset, *Callithrix (Mico) manicorensis*; Silvery Marmoset, *Callithrix (Mico) argentata*; White Marmoset, *Callithrix (Mico) leucippe*; Emilia's Marmoset, *Callithrix (Mico) emiliae*; Black-headed Marmoset, *Callithrix (Mico) nigriceps*; Marca's Marmoset, *Callithrix (Mico) marcai*; Black-tailed Marmoset, *Callithrix (Mico) melanura*; Santarem Marmoset, *Callithrix (Mico) humeralifera*; Maués Marmoset, *Callithrix (Mico) mauesi*; Gold-and-white Marmoset, *Callithrix (Mico) chrysoleuca*; Hershkovitz's Marmoset, *Callithrix (Mico) intermedia*; Satéré Marmoset, *Callithrix (Mico) saterei*); Roosmalens' Dwarf Marmoset belonging to the subgenus *Callibella (Callithrix (Callibella) humilis*); or the Pygmy Marmoset belonging to the subgenus *Cebuella (Callithrix (Cebuella) pygmaea*).

Other genera of the New-World Monkeys comprise tamarins of the genus *Saguinus* (comprising the *S. oedipus*-group, the *S. midas* group, the *S. nigricollis* group, the *S. mystax* group, the *S. bicolor* group and the *S. inustus* group) and squirrel monkeys of the genus *Saimiri* (e.g. *Saimiri sciureus*, *Saimiri oerstedii*, *Saimiri ustus*, *Saimiri boliviensis*, *Saimiri vanzolini*).

Advantageously, the present invention provides also target antigenxCD3 bispecific single chain antibodies comprising a second binding domain which binds both to the human target antigen and to the macaque target antigen homolog, i.e. the homolog of a non-chimpanzee primate. In a preferred embodiment, the bispecific single chain antibody thus comprises a second binding domain exhibiting cross-species specificity to the human and a non-chimpanzee primate target antigen. In this case, the identical bispecific single chain antibody molecule can be used both for preclinical

evaluation of safety, activity and/or pharmacokinetic profile of these binding domains in primates and as drug in humans. Put in other words, the same molecule can be used in preclinical animal studies as well as in clinical studies in humans. This leads to highly comparable results and a much-increased predictive power of the animal studies compared to species-specific surrogate molecules. Since both the CD3 and the target antigen binding domain of the target antigenxCD3 bispecific single chain antibody of the invention are cross-species specific, i.e. reactive with the human and non-chimpanzee primates' antigens, it can be used both for preclinical evaluation of safety, activity and/or pharmacokinetic profile of these binding domains in primates and – in the identical form - as drug in humans. It will be understood that in a preferred embodiment, the cross-species specificity of the first and second binding domain of the antibodies of the invention is identical.

It has been found in the present invention that it is possible to generate a, preferably human, target antigenxCD3 bispecific single chain antibody wherein the identical molecule can be used in preclinical animal testing, as well as clinical studies and even in therapy in human. This is due to the unexpected identification of the, preferably human, target antigenxCD3 bispecific single chain antibody, which, in addition to binding to human CD3 epsilon and target antigen, respectively, (and due to genetic similarity likely to the chimpanzee counterpart), also binds to the homologs of said antigens of non-chimpanzee primates, including New-World Monkeys and Old-World Monkeys. The preferably human, target antigenxCD3 bispecific single chain antibody of the invention can be used as therapeutic agent against various diseases, including, but not limited, to cancer. In view of the above, the need to construct a surrogate target antigenxCD3 bispecific single chain antibody for testing in a phylogenetic distant (from humans) species disappears. As a result, the identical molecule can be used in animal preclinical testing as is intended to be administered to humans in clinical testing as well as following market approval and therapeutic drug administration. The ability to use the same molecule for preclinical animal testing as in later administration to humans virtually eliminates, or at least greatly reduces, the danger that the data obtained in preclinical animal testing have limited applicability to the human case. In short, obtaining preclinical safety data in animals using the same molecule as will actually be administered to humans does much to

ensure the applicability of the data to a human-relevant scenario. In contrast, in conventional approaches using surrogate molecules, said surrogate molecules have to be molecularly adapted to the animal test system used for preclinical safety assessment. Thus, the molecule to be used in human therapy in fact differs in sequence and also likely in structure from the surrogate molecule used in preclinical testing in pharmacokinetic parameters and/or biological activity, with the consequence that data obtained in preclinical animal testing have limited applicability / transferability to the human case. The use of surrogate molecules requires the construction, production, purification and characterization of a completely new construct. This leads to additional development costs and time necessary to obtain that molecule. In sum, surrogates have to be developed separately in addition to the actual drug to be used in human therapy, so that two lines of development for two molecules have to be carried out. Therefore, a major advantage of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention exhibiting cross-species specificity described herein is that the identical molecule can be used for therapeutic agents in humans and in preclinical animal testing.

It is preferred that at least one of said first or second binding domains of the bispecific single chain antibody of the invention is CDR-grafted, humanized or human, as set forth in more detail below. Preferably, both the first and second binding domains of the bispecific single chain antibody of the invention are CDR-grafted, humanized or human. For the preferably human, target antigenxCD3 bispecific single chain antibody of the invention, the generation of an immune reaction against said binding molecule is excluded to the maximum possible extent upon administration of the molecule to human patients.

Another major advantage of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention is its applicability for preclinical testing in various primates. The behavior of a drug candidate in animals should ideally be indicative of the expected behavior of this drug candidate upon administration to humans. As a result, the data obtained from such preclinical testing should therefore generally have a highly predictive power for the human case. However, as learned from the tragic outcome of the recent Phase I clinical trial on TGN1412 (a CD28 monoclonal antibody), a drug candidate may act differently in a primate species than

in humans: Whereas in preclinical testing of said antibody no or only limited adverse effects have been observed in animal studies performed with cynomolgus monkeys, six human patients developed multiple organ failure upon administration of said antibody (Lancet 368 (2006), 2206-7). The results of these dramatic, non-desired
5 negative events suggest that it may not be sufficient to limit preclinical testing to only one (non-chimpanzee primate) species. The fact that the target antigenxCD3 bispecific single chain antibody of the invention binds to a series of New-World and Old-World Monkeys may help to overcome the problems faced in the case mentioned above. Accordingly, the present invention provides means and methods for
10 minimizing species differences in effects when drugs for human therapy are being developed and tested.

With the, preferably human, cross-species specific target antigenxCD3 bispecific single chain antibody of the invention it is also no longer necessary to adapt the test
15 animal to the drug candidate intended for administration to humans, such as e.g. the creation of transgenic animals. The, preferably human, target antigenxCD3 bispecific single chain antibody of the invention exhibiting cross-species specificity according to the uses and the methods of invention can be directly used for preclinical testing in non-chimpanzee primates, without any genetic manipulation of the animals. As well
20 known to those skilled in the art, approaches in which the test animal is adapted to the drug candidate always bear the risk that the results obtained in the preclinical safety testing are less representative and predictive for humans due to the modification of the animal. For example, in transgenic animals, the proteins encoded by the transgenes are often highly over-expressed. Thus, data obtained for the
25 biological activity of an antibody against this protein antigen may be limited in their predictive value for humans in which the protein is expressed at much lower, more physiological levels.

A further advantage of the uses of the preferably human target antigenxCD3
30 bispecific single chain antibody of the invention exhibiting cross-species specificity is the fact that chimpanzees as an endangered species are avoided for animal testing. Chimpanzees are the closest relatives to humans and were recently grouped into the family of hominids based on the genome sequencing data (Wildman et al., PNAS 100 (2003), 7181). Therefore, data obtained with chimpanzee is generally considered to

be highly predictive for humans. However, due to their status as endangered species, the number of chimpanzees, which can be used for medical experiments, is highly restricted. As stated above, maintenance of chimpanzees for animal testing is therefore both costly and ethically problematic. The uses of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention avoid both ethical objections and financial burden during preclinical testing without prejudicing the quality, i.e. applicability, of the animal testing data obtained. In light of this, the uses of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention provide for a reasonable alternative for studies in chimpanzees.

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A still further advantage of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention is the ability of extracting multiple blood samples when using it as part of animal preclinical testing, for example in the course of pharmacokinetic animal studies. Multiple blood extractions can be much more readily obtained with a non-chimpanzee primate than with lower animals, e.g. a mouse. The extraction of multiple blood samples allows continuous testing of blood parameters for the determination of the biological effects induced by the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention. Furthermore, the extraction of multiple blood samples enables the researcher to evaluate the pharmacokinetic profile of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention as defined herein. In addition, potential side effects, which may be induced by said, preferably human, target antigenxCD3 bispecific single chain antibody of the invention reflected in blood parameters can be measured in different blood samples extracted during the course of the administration of said antibody. This allows the determination of the potential toxicity profile of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention as defined herein.

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The advantages of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention as defined herein exhibiting cross-species specificity may be briefly summarized as follows:

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First, the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention as defined herein used in preclinical testing is the same as the one used in human therapy. Thus, it is no longer necessary to develop two independent

molecules, which may differ in their pharmacokinetic properties and biological activity. This is highly advantageous in that e.g. the pharmacokinetic results are more directly transferable and applicable to the human setting than e.g. in conventional surrogate approaches.

- 5 Second, the uses of the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention as defined herein for the preparation of therapeutics in human is less cost- and labor-intensive than surrogate approaches.

Third, the, preferably human, target antigenxCD3 bispecific single chain antibody of the invention as defined herein can be used for preclinical testing not only in one
10 primate species, but in a series of different primate species, thereby limiting the risk of potential species differences between primates and human.

Fourth, chimpanzee as an endangered species for animal testing can be avoided if desired.

Fifth, multiple blood samples can be extracted for extensive pharmacokinetic studies.

- 15 Sixth, due to the human origin of the, preferably human, binding molecules according to a preferred embodiment of the invention, the generation of an immune reaction against said binding molecules is minimized when administered to human patients. Induction of an immune response with antibodies specific for a drug candidate derived from a non-human species as e.g. a mouse leading to the development of
20 human-anti-mouse antibodies (HAMAs) against therapeutic molecules of murine origin is excluded.

Last but not least, the therapeutic use of the target antigenxCD3 bispecific single chain antibody of the invention provides a novel and inventive therapeutic approach for cancer, preferably solid tumors, more preferably carcinomas and prostate cancer.

- 25 As shown in the following examples, the target antigenxCD3 bispecific single chain antibody of the invention provides an advantageous tool in order to kill target antigen-expressing human target cells, e.g. cancer cells. Moreover, the cytotoxic activity of the target antigenxCD3 bispecific single chain antibody of the invention is higher than the activity of antibodies described in the art for the exemplified targets.

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It is further preferred for the method of the invention that the first binding domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from:

- (a) CDR-L1 as depicted in SEQ ID NO. 27, CDR-L2 as depicted in SEQ ID NO. 28 and CDR-L3 as depicted in SEQ ID NO. 29;
- (b) CDR-L1 as depicted in SEQ ID NO. 117, CDR-L2 as depicted in SEQ ID NO. 118 and CDR-L3 as depicted in SEQ ID NO. 119; and
- 5 (c) CDR-L1 as depicted in SEQ ID NO. 153, CDR-L2 as depicted in SEQ ID NO. 154 and CDR-L3 as depicted in SEQ ID NO. 155.

More preferably, the first binding domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises a VL region selected from the group consisting of a VL region as depicted in SEQ ID NO. 35, 39, 125, 129, 161 or
10 165.

It is alternatively preferred for the method of the invention that the first binding domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises a VH region comprising CDR-H 1, CDR-H2 and CDR-H3 selected from:

- 15 (a) CDR-H1 as depicted in SEQ ID NO. 12, CDR-H2 as depicted in SEQ ID NO. 13 and CDR-H3 as depicted in SEQ ID NO. 14;
- (b) CDR-H1 as depicted in SEQ ID NO. 30, CDR-H2 as depicted in SEQ ID NO. 31 and CDR-H3 as depicted in SEQ ID NO. 32;
- (c) CDR-H1 as depicted in SEQ ID NO. 48, CDR-H2 as depicted in SEQ ID NO. 49
20 and CDR-H3 as depicted in SEQ ID NO. 50;
- (d) CDR-H1 as depicted in SEQ ID NO. 66, CDR-H2 as depicted in SEQ ID NO. 67 and CDR-H3 as depicted in SEQ ID NO. 68;
- (e) CDR-H1 as depicted in SEQ ID NO. 84, CDR-H2 as depicted in SEQ ID NO. 85 and CDR-H3 as depicted in SEQ ID NO. 86;
- 25 (f) CDR-H1 as depicted in SEQ ID NO. 102, CDR-H2 as depicted in SEQ ID NO. 103 and CDR-H3 as depicted in SEQ ID NO. 104;
- (g) CDR-H1 as depicted in SEQ ID NO. 120, CDR-H2 as depicted in SEQ ID NO. 121 and CDR-H3 as depicted in SEQ ID NO. 122;
- (h) CDR-H1 as depicted in SEQ ID NO. 138, CDR-H2 as depicted in SEQ ID NO. 139 and CDR-H3 as depicted in SEQ ID NO. 140;
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- (i) CDR-H1 as depicted in SEQ ID NO. 156, CDR-H2 as depicted in SEQ ID NO. 157 and CDR-H3 as depicted in SEQ ID NO. 158; and
- (j) CDR-H1 as depicted in SEQ ID NO. 174, CDR-H2 as depicted in SEQ ID NO. 175 and CDR-H3 as depicted in SEQ ID NO. 176.

More preferably, the binding domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises a VH region selected from the group consisting of a VH region as depicted in SEQ ID NO. 15, 19, 33, 37, 51, 55, 69, 73, 87, 91, 105, 109, 123, 127, 141, 145, 159, 163, 177 or 181.

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It is preferred for the method of the invention that the first binding domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises a VL region and a VH region selected from the group consisting of:

- (a) a VL region as depicted in SEQ ID NO. 17 or 21 and a VH region as depicted in
10 SEQ ID NO. 15 or 19;
- (b) a VL region as depicted in SEQ ID NO. 35 or 39 and a VH region as depicted in
SEQ ID NO. 33 or 37;
- (c) a VL region as depicted in SEQ ID NO. 53 or 57 and a VH region as depicted in
SEQ ID NO. 51 or 55;
- 15 (d) a VL region as depicted in SEQ ID NO. 71 or 75 and a VH region as depicted in
SEQ ID NO. 69 or 73;
- (e) a VL region as depicted in SEQ ID NO. 89 or 93 and a VH region as depicted in
SEQ ID NO. 87 or 91;
- (f) a VL region as depicted in SEQ ID NO. 107 or 111 and a VH region as depicted
20 in SEQ ID NO. 105 or 109;
- (g) a VL region as depicted in SEQ ID NO. 125 or 129 and a VH region as depicted
in SEQ ID NO. 123 or 127;
- (h) a VL region as depicted in SEQ ID NO. 143 or 147 and a VH region as depicted
in SEQ ID NO. 141 or 145;
- 25 (i) a VL region as depicted in SEQ ID NO. 161 or 165 and a VH region as depicted
in SEQ ID NO. 159 or 163; and
- (j) a VL region as depicted in SEQ ID NO. 179 or 183 and a VH region as depicted
in SEQ ID NO. 177 or 181.

More preferably, the first binding domain capable of binding to an epitope of human
30 and non-chimpanzee primate CD3 ϵ chain comprises an amino acid sequence
selected from the group consisting of SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95,
97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187.

As already discussed herein above, it is also preferred for the method of the invention that also the second binding domain binds to epitopes/binding sites in the extracellular domain of a high molecular weight antigen of human and non-chimpanzee primate.

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In a preferred embodiment of the method of the invention the second binding domain binds to epitopes/binding sites in the extracellular domain of c-MET. This target antigen and its expression characteristics have been described herein above. The MET tyrosine kinase receptor with an extracellular region of 908 aa with the following sequential arrangement of independently folded extracellular domains from membrane-proximal to membrane-distal: Four Ig domains of together 362 aa (residues 563–924), a cystein-rich domain of 42 aa (residues 520-561), the beta-chain of a sema domain of 212 aa (residues 308-519) and the alpha-chain of the sema domain of 282 aa (residues 25-307). Accordingly, it is preferred for the method of the invention, that the second binding domain binds to epitopes/binding sites in the four Ig domains (SEQ ID NO: 436), a cystein-rich domain (SEQ ID NO: 437), or the beta-chain of a sema domain (SEQ ID NO: 438) of the extracellular domain of c-MET, which are all below the 640 aa-threshold.

In an alternatively preferred embodiment of the method of the invention the second binding domain binds to epitopes/binding sites in the extracellular domain of endosialin (TEM1). This target antigen and its expression characteristics have been described herein above. For endosialin a extracellular domain consisting of 665 aa and the following sequential arrangement of independently folded extracellular domains from membrane-proximal to membrane-distal is described in the art: a mucin domain of 326 aa (residues 360–685), three EGF-like domains of together 116 aa (residues 235-350), a Sushi/SCR/CCP domain of 55 aa (residues 176-230) and a C-type lectin domain of 129 aa (residues 29-157). Accordingly, it is preferred for the method of the invention that the second binding domain binds to epitopes/binding sites in the mucin domain (SEQ ID NO: 440), the three EGF-like domains (SEQ ID NO: 441), or the Sushi/SCR/CCP domain (SEQ ID NO: 442) of the extracellular domain of TEM1.

According to a further alternatively preferred embodiment of the method of the invention the second binding domain binds to epitopes/binding sites in the extracellular domain of IGF-1R. This target antigen and its expression characteristics

have been described herein above. For endosialin a extracellular domain consisting of 905 aa and the following sequential arrangement of independently folded extracellular domains from membrane-proximal to membrane-distal is described in the art: three fibronectin type III domains of together 447 aa (residues 460–906), an L2 domain of 160 aa (residues 300–459), a cystein-rich domain of 149 aa (residues 151–299) and an L1 domain of 150 aa (residues 1–150). Accordingly, it is preferred for the method of the invention that the second binding domain binds to epitopes/binding sites in the three fibronectin type III domains (SEQ ID NO: 444), and the L2 domain (SEQ ID NO: 445) of the extracellular domain of IGF-1R.

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An alternative embodiment of the invention relates to a bispecific single chain antibody comprising a first domain binding domain capable of binding to CD3 epsilon (CD3 ϵ) of human and non-chimpanzee primate and a second domain binding domain capable of binding to the extracellular domain of the mutated human PSMA having an amino acid sequence as depicted in SEQ ID NO: 447 but not to the extracellular domain of the rodent PSMA. In other words, the bispecific antibody of the invention specifically binds to membrane proximal epitopes, i.e. epitopes formed only by amino acid residues of the extracellular domain of PSMA, the alpha C-atom of which has a distance of less than 60Å from the reference C-atom (the alpha C-atom of the 13th aa as counted from the junction of transmembrane and extracellular region). The specific superior characteristics of these PSMAxCD3 bispecific single chain antibodies have been described herein above. Moreover, corresponding antibodies are exemplified and characterized in the appended examples.

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It is preferred for the bispecific single chain antibody comprising a first domain binding domain capable of binding to CD3 epsilon (CD3 ϵ) and a second domain binding domain capable of binding to the extracellular domain of the mutated human PSMA having an amino acid sequence as depicted in SEQ ID NO: 447 but not to the extracellular domain of the rodent PSMA that the first domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187.

It is preferred for the bispecific single chain antibodies that the second domain comprises a VL region comprising CDR-L1, CDR-L2 and CDR-L3 selected from:

- (a) CDR-L1 as depicted in SEQ ID NO. 269, CDR-L2 as depicted in SEQ ID NO: 270 and CDR-L3 as depicted in SEQ ID NO. 271;
- (b) CDR-L1 as depicted in SEQ ID NO. 283, CDR-L2 as depicted in SEQ ID NO: 284 and CDR-L3 as depicted in SEQ ID NO. 285;
- 5 (c) CDR-L1 as depicted in SEQ ID NO. 297, CDR-L2 as depicted in SEQ ID NO: 298 and CDR-L3 as depicted in SEQ ID NO. 299;
- (d) CDR-L1 as depicted in SEQ ID NO. 311, CDR-L2 as depicted in SEQ ID NO: 312 and CDR-L3 as depicted in SEQ ID NO. 313;
- (e) CDR-L1 as depicted in SEQ ID NO. 325, CDR-L2 as depicted in SEQ ID NO.
10 326 and CDR-L3 as depicted in SEQ ID NO. 327;
- (f) CDR-L1 as depicted in SEQ ID NO. 255, CDR-L2 as depicted in SEQ ID NO. 256 and CDR-L3 as depicted in SEQ ID NO. 257; and
- (g) CDR-L1 as depicted in SEQ ID NO. 481, CDR-L2 as depicted in SEQ ID NO. 482 and CDR-L3 as depicted in SEQ ID NO. 483.
- 15 It is also preferred for the bispecific single chain antibodies of the invention that the second domain comprises a VH region comprising CDR-H1, CDR-H2 and CDR-H3 selected from:
 - (a) CDR-H1 as depicted in SEQ ID NO. 274, CDR-H2 as depicted in SEQ ID NO: 275 and CDR-H3 as depicted in SEQ ID NO. 276;
 - 20 (b) CDR-H1 as depicted in SEQ ID NO. 288, CDR-H2 as depicted in SEQ ID NO: 289 and CDR-H3 as depicted in SEQ ID NO. 290;
 - (c) CDR-H1 as depicted in SEQ ID NO. 302, CDR-H2 as depicted in SEQ ID NO: 303 and CDR-H3 as depicted in SEQ ID NO. 304;
 - (d) CDR-H1 as depicted in SEQ ID NO. 316, CDR-H2 as depicted in SEQ ID NO:
25 317 and CDR-H3 as depicted in SEQ ID NO. 318;
 - (e) CDR-H1 as depicted in SEQ ID NO. 330, CDR-H2 as depicted in SEQ ID NO: 331 and CDR-H3 as depicted in SEQ ID NO. 332;
 - (f) CDR-H1 as depicted in SEQ ID NO. 260, CDR-H2 as depicted in SEQ ID NO: 261 and CDR-H3 as depicted in SEQ ID NO. 262; and
 - 30 (g) CDR-H1 as depicted in SEQ ID NO. 476, CDR-H2 as depicted in SEQ ID NO: 477 and CDR-H3 as depicted in SEQ ID NO. 478.

In a further preferred embodiment of a bispecific single chain antibody of the invention the second domain comprises a VL region and a VH region selected from the group consisting of:

- 5 (a) a VL region as depicted in SEQ ID NO. 268 and a VH region as depicted in SEQ ID NO. 273;
- (b) a VL region as depicted in SEQ ID NO. 282 and a VH region as depicted in SEQ ID NO. 287;
- (c) a VL region as depicted in SEQ ID NO. 296 and a VH region as depicted in SEQ ID NO. 301;
- 10 (d) a VL region as depicted in SEQ ID NO. 310 and a VH region as depicted in SEQ ID NO. 315;
- (e) a VL region as depicted in SEQ ID NO. 324 and a VH region as depicted in SEQ ID NO. 329;
- (f) a VL region as depicted in SEQ ID NO. 254 and a VH region as depicted in
15 SEQ ID NO. 259; and
- (g) a VL region as depicted in SEQ ID NO. 480 and a VH region as depicted in SEQ ID NO. 475.

More preferably, the second domain comprises an amino acid sequence selected
20 from the group consisting of SEQ ID NOs: 278, 292, 306, 320, 334, 485 or 264.

It is preferred for the bispecific single chain antibody comprising a first domain binding domain capable of binding to CD3 epsilon (CD3 ϵ) of human and non-chimpanzee primate and a second domain binding domain capable of binding to the extracellular domain of the mutated human PSMA chimera that the first domain
25 capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187.

A particularly preferred embodiment of the invention concerns an above
30 characterized polypeptide, wherein the bispecific single chain antibody molecule comprises a sequence selected from:

- (a) an amino acid sequence as depicted in any of SEQ ID NOs. 280, 294, 308, 322, 336, 266 or 487;

- (b) an amino acid sequence encoded by a nucleic acid sequence as depicted in any of SEQ ID NOs: 281, 295, 309, 267, 323, 337 or 488; and
- (c) an amino acid sequence at least 90 % identical, more preferred at least 95 % identical, most preferred at least 96 % identical to the amino acid sequence of (a) or (b).

The invention relates to a bispecific single chain antibody molecule comprising an amino acid sequence as depicted in any of SEQ ID NOs: 280, 294, 266, 308, 322, 336 or 487, as well as to an amino acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 % identical, most preferred at least 96, 97, 98, or 99 % identical to the amino acid sequence of SEQ ID NOs: 280, 294, 266, 308, 322, 336 or 487. The invention relates also to the corresponding nucleic acid sequences as depicted in any of SEQ ID NOs: 281, 295, 267, 309, 323, 337 or 488, as well as to nucleic acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 % identical, most preferred at least 96, 97, 98, or 99 % identical to the nucleic acid sequences shown in SEQ ID NOs: 281, 295, 267, 309, 323, 337 or 488.

Preferred domain arrangements in the PSMAxCD3 bispecific single chain antibody constructs of the invention are shown in the following examples.

In a preferred embodiment of the invention, the bispecific single chain antibodies are cross-species specific for CD3 epsilon and for the human and non-chimpanzee primate cell surface antigen PSMA, recognized by their second binding domain.

In an alternative embodiment the invention provides a bispecific single chain antibody comprising a first domain binding domain capable of binding to CD3 epsilon (CD3 ϵ) of human and non-chimpanzee primate and a second domain binding domain capable of binding to the extracellular domain of the mutated human FAP α chimera having an amino acid sequence as depicted in SEQ ID NO: 448 but not to the extracellular domain of the rodent FAP α . In other words, the bispecific antibody of the invention specifically binds to membrane proximal epitopes, i.e. epitopes formed only by amino acid residues of the extracellular domain of FAP α , the alpha C-atom of which has a distance of less than 60Å from the reference C-atom (the alpha C-atom of the 13th aa as counted from the junction of transmembrane and extracellular region).

According to a preferred embodiment of the invention an above characterized bispecific single chain antibody molecule comprises a group of the following

sequences as CDR H1, CDR H2, CDR H3, CDR L1, CDR L2 and CDR L3 in the second binding domain selected from:

CDR H1-3 of SEQ ID NO: 1137 - 1139 and CDR L1-3 of SEQ ID NO: 1132 - 1134.

- 5 The sequences of the corresponding VL- and VH-regions of the second binding domain of the bispecific single chain antibody molecule of the invention as well as of the respective scFvs are shown in the sequence listing.

According to a preferred embodiment of the invention an above characterized bispecific single chain antibody molecule comprises a group of the following
10 sequences as CDR H1, CDR H2, CDR H3, CDR L1, CDR L2 and CDR L3 in the second binding domain selected from the group consisting of:

- a) CDR H1-3 of SEQ ID NO: 808 - 810 and CDR L1-3 of SEQ ID NO: 813 - 815;
- b) CDR H1-3 of SEQ ID NO: 794 - 796 and CDR L1-3 of SEQ ID NO: 799 -
15 801;
- c) CDR H1-3 of SEQ ID NO: 738 - 740 and CDR L1-3 of SEQ ID NO: 743 - 745;
- d) CDR H1-3 of SEQ ID NO: 752 - 754 and CDR L1-3 of SEQ ID NO: 757 - 759;
- e) CDR H1-3 of SEQ ID NO: 822 - 824 and CDR L1-3 of SEQ ID NO: 827 -
20 829;
- f) CDR H1-3 of SEQ ID NO: 766 - 768 and CDR L1-3 of SEQ ID NO: 771 - 773; and
- g) CDR H1-3 of SEQ ID NO: 780 - 782 and CDR L1-3 of SEQ ID NO: 785 -
25 787.

In the bispecific single chain antibody molecule of the invention the binding domains are arranged in the order VL-VH-VH-VL, VL-VH-VL-VH, VH-VL-VH-VL or VH-VL-VL-VH, as exemplified in the appended examples. Preferably, the binding domains are arranged in the order VH FAP alpha -VL FAP alpha -VH CD3-VL CD3 or VL FAP
30 alpha -VH FAP alpha -VH CD3-VL CD3. More preferred, the binding domains are arranged in the order VL FAP alpha -VH FAP alpha -VH CD3-VL CD3.

It is preferred for the bispecific single chain antibody comprising a first domain binding domain capable of binding to CD3 epsilon (CD3ε) of human and non-chimpanzee primate and a second domain binding domain capable of binding to the

extracellular domain of the mutated human FAP α chimera that the first domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187.

A particularly preferred embodiment of the invention concerns an above characterized polypeptide, wherein the bispecific single chain antibody molecule comprises a sequence selected from:

- (a) an amino acid sequence as depicted in any of SEQ ID NOs: 819, 805, 749, 763, 833, 777 or 791;
- (b) an amino acid sequence encoded by a nucleic acid sequence as depicted in any of SEQ ID NOs: 820, 806, 750, 764, 834, 778 or 792; and
- (c) an amino acid sequence at least 90 % identical, more preferred at least 95 % identical, most preferred at least 96 % identical to the amino acid sequence of (a) or (b).

The invention relates to a bispecific single chain antibody molecule comprising an amino acid sequence as depicted in any of SEQ ID NOs: 819, 805, 749, 763, 833, 777 or 791, as well as to an amino acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 % identical, most preferred at least 96, 97, 98, or 99 % identical to the amino acid sequence of SEQ ID NOs: 819, 805, 749, 763, 833, 777 or 791. The invention relates also to the corresponding nucleic acid sequences as depicted in any of SEQ ID NOs: 820, 806, 750, 764, 834, 778 or 792, as well as to nucleic acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 % identical, most preferred at least 96, 97, 98, or 99 % identical to the nucleic acid sequences shown in SEQ ID NOs: 820, 806, 750, 764, 834, 778 or 792.

Preferred domain arrangements in the FAP α xCD3 bispecific single chain antibody constructs of the invention are shown in the following examples.

In a further alternative embodiment the invention provides a bispecific single chain antibody comprising a first domain binding domain capable of binding to CD3 epsilon (CD3 ϵ) of human and non-chimpanzee primate and a second domain binding domain capable of binding to the four Ig domains (SEQ ID NO: 436), a cystein-rich domain (SEQ ID NO: 437), or the beta-chain of a sema domain (SEQ ID NO: 438) of the extracellular domain of c-MET.

According to a preferred embodiment of the invention an above characterized bispecific single chain antibody molecule comprises a group of the following sequences as CDR H1, CDR H2, CDR H3, CDR L1, CDR L2 and CDR L3 in the second binding domain selected from the group consisting of:

- 5 a) CDR H1-3 of SEQ ID NO: -500 - 502 and CDR L1-3 of SEQ ID NO: -505 - 507;
- b) CDR H1-3 of SEQ ID NO: 514 - 516 and CDR L1-3 of SEQ ID NO: 519 - 521;
- c) CDR H1-3 of SEQ ID NO: 528 - 530 and CDR L1-3 of SEQ ID NO: 533 - 535;
- 10 d) CDR H1-3 of SEQ ID NO: 542 - 544 and CDR L1-3 of SEQ ID NO: 547 - 549;
- e) CDR H1-3 of SEQ ID NO: 556 - 558 and CDR L1-3 of SEQ ID NO: 561 - 563;
- 15 f) CDR H1-3 of SEQ ID NO: 570 - 572 and CDR L1-3 of SEQ ID NO: 575 - 577;
- g) CDR H1-3 of SEQ ID NO: 584 - 586 and CDR L1-3 of SEQ ID NO: 589 - 591;
- h) CDR H1-3 of SEQ ID NO: 598 - 600 and CDR L1-3 of SEQ ID NO: 603 - 605;
- 20 i) CDR H1-3 of SEQ ID NO: 612 - 614 and CDR L1-3 of SEQ ID NO: 617 - 619;
- j) CDR H1-3 of SEQ ID NO: 626 - 628 and CDR L1-3 of SEQ ID NO: 631 - 633;
- 25 k) CDR H1-3 of SEQ ID NO: 640 - 642 and CDR L1-3 of SEQ ID NO: 645 - 647;
- l) CDR H1-3 of SEQ ID NO: 654 - 656 and CDR L1-3 of SEQ ID NO: 659 - 661;
- m) CDR H1-3 of SEQ ID NO: 668 - 670 and CDR L1-3 of SEQ ID NO: 673 - 675;
- 30 n) CDR H1-3 of SEQ ID NO: 682 - 684 and CDR L1-3 of SEQ ID NO: 687 - 689;
- o) CDR H1-3 of SEQ ID NO: 696 - 698 and CDR L1-3 of SEQ ID NO: 701 - 703;

p) CDR H1-3 of SEQ ID NO: 710 - 712 and CDR L1-3 of SEQ ID NO: 715 - 717; and

q) CDR H1-3 of SEQ ID NO: 724 - 726 and CDR L1-3 of SEQ ID NO: 729 - 731.

5 The sequences of the corresponding VL- and VH-regions of the second binding domain of the bispecific single chain antibody molecule of the invention as well as of the respective scFvs are shown in the sequence listing.

In the bispecific single chain antibody molecule of the invention the binding domains are arranged in the order VL-VH-VH-VL, VL-VH-VL-VH, VH-VL-VH-VL or VH-VL-VL-VH, as exemplified in the appended examples. Preferably, the binding domains are arranged in the order VH C-MET-VL C-MET-VH CD3-VL CD3 or VL C-MET-VH C-MET-VH CD3-VL CD3.

More preferably, the first domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187.

A particularly preferred embodiment of the invention concerns an above characterized polypeptide, wherein the bispecific single chain antibody molecule comprises a sequence selected from:

- 20 (a) an amino acid sequence as depicted in any of SEQ ID NOs. 511, 525, 539, 553, 567 or 581;
- (b) an amino acid sequence encoded by a nucleic acid sequence as depicted in any of SEQ ID NOs: 512, 526, 540, 554, 568 or 582; and
- (c) an amino acid sequence at least 90 % identical, more preferred at least 95 % identical, most preferred at least 96 % identical to the amino acid sequence of (a) or (b).

The invention relates to a bispecific single chain antibody molecule comprising an amino acid sequence as depicted in any of SEQ ID NOs: 511, 525, 539, 553, 567 or 581, as well as to an amino acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 % identical, most preferred at least 96, 97, 98, or 99 % identical to the amino acid sequence of SEQ ID NOs: 511, 525, 539, 553, 567 or 581. The invention relates also to the corresponding nucleic acid sequences as depicted in any of SEQ ID NOs: 512, 526, 540, 554, 568 or 582 as well as to nucleic acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 %

identical, most preferred at least 96, 97, 98, or 99 % identical to the nucleic acid sequences shown in SEQ ID NOs: 512, 526, 540, 554, 568 or 582.

Preferred domain arrangements in the c-METxCD3 bispecific single chain antibody constructs of the invention are shown in the following examples.

5

According to an alternative embodiment the invention provides a bispecific single chain antibody comprising a first domain binding domain capable of binding to CD3 epsilon (CD3 ϵ) of human and non-chimpanzee primate and a second domain binding domain capable of binding to the mucin domain (SEQ ID NO: 440), the three EGF-like domains (SEQ ID NO: 441), or the Sushi/SCR/CCP domain (SEQ ID NO: 442) of the extracellular domain of endosialin (TEM1).

10

Preferably the first domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187.

15

Moreover, in an alternative embodiment the invention provides a bispecific single chain antibody comprising a first domain binding domain capable of binding to CD3 epsilon (CD3 ϵ) of human and non-chimpanzee primate and a second domain binding domain capable of binding to the three fibronectin type III domains (SEQ ID NO: 444), the L2 domain (SEQ ID NO: 445) of the extracellular domain of IGF-1R.

20

According to a preferred embodiment of the invention an above characterized bispecific single chain antibody molecule comprises a group of the following sequences as CDR H1, CDR H2, CDR H3, CDR L1, CDR L2 and CDR L3 in the second binding domain selected from the group consisting of:

25

a) CDR H1-3 of SEQ ID NO: -836 - 838 and CDR L1-3 of SEQ ID NO: -841 - 843; and

b) CDR H1-3 of SEQ ID NO: 850 - 852 and CDR L1-3 of SEQ ID NO: 855 - 857.

30

The sequences of the corresponding VL- and VH-regions of the second binding domain of the bispecific single chain antibody molecule of the invention as well as of the respective scFvs are shown in the sequence listing.

In the bispecific single chain antibody molecule of the invention the binding domains are arranged in the order VL-VH-VH-VL, VL-VH-VL-VH, VH-VL-VH-VL or VH-VL-VL-

VH, as exemplified in the appended examples. Preferably, the binding domains are arranged in the order VH IGF-1R-VL IGF-1R-VH CD3-VL CD3 or VL IGF-1R-VH IGF-1R-VH CD3-VL CD3.

Preferably, the first domain capable of binding to an epitope of human and non-chimpanzee primate CD3 ϵ chain comprises an amino acid sequence selected from the group consisting of SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187.

A particularly preferred embodiment of the invention concerns an above characterized polypeptide, wherein the bispecific single chain antibody molecule comprises a sequence selected from:

- (a) an amino acid sequence as depicted in any of SEQ ID NOs: 847 or 861;
- (b) an amino acid sequence encoded by a nucleic acid sequence as depicted in any of SEQ ID NOs: 848, or 862; and
- (c) an amino acid sequence at least 90 % identical, more preferred at least 95 % identical, most preferred at least 96 % identical to the amino acid sequence of (a) or (b).

The invention relates to a bispecific single chain antibody molecule comprising an amino acid sequence as depicted in any of SEQ ID NOs: 847 or 861, as well as to an amino acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 % identical, most preferred at least 96, 97, 98, or 99 % identical to the amino acid sequence of SEQ ID NOs: 847 or 861. The invention relates also to the corresponding nucleic acid sequences as depicted in any of SEQ ID NOs: 848, or 862 as well as to nucleic acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 % identical, most preferred at least 96, 97, 98, or 99 % identical to the nucleic acid sequences shown in SEQ ID NOs: 848, or 862.

Preferred domain arrangements in the IGF-1RxCD3 bispecific single chain antibody constructs of the invention are shown in the following examples.

The invention relates to bispecific single chain antibody molecule comprising the above identified amino acid sequences, as well as to amino acid sequences at least 85% identical, preferably 90 %, more preferred at least 95 % identical, most preferred at least 96, 97, 98, or 99 % identical to the amino acid sequence of said sequences. As described herein above, the specificity of an antibody is generally understood to

be determined by the CDR sequences. Accordingly, in order to maintain the specificity of a given group of CDRs, e.g. three CDRs of a heavy chain and three CDRs of a light chain, the sequence of these CDRs has to be conserved. Accordingly variants of bispecific single antibodies as identified herein, which also fall under the present invention are preferably variants having more than one amino acid substituions in FR regions instead of the CDRs. It is to be understood that the sequence identity is determined over the entire nucleotide or amino acid sequence. For sequence alignments, for example, the programs Gap or BestFit can be used (Needleman and Wunsch J. Mol. Biol. 48 (1970), 443-453; Smith and Waterman, Adv. Appl. Math 2 (1981), 482-489), which is contained in the GCG software package (Genetics Computer Group, 575 Science Drive, Madison, Wisconsin, USA 53711 (1991). It is a routine method for those skilled in the art to determine and identify a nucleotide or amino acid sequence having e.g. 85% (90%, 95%, 96%, 97%, 98% or 99%) sequence identity to the nucleotide or amino acid sequences of the bispecific single chain antibody of the invention by using e.g. one of the above mentioned programs. For example, according to Crick's Wobble hypothesis, the 5' base on the anti-codon is not as spatially confined as the other two bases, and could thus have non-standard base pairing. Put in other words: the third position in a codon triplet may vary so that two triplets which differ in this third position may encode the same amino acid residue. Said hypothesis is well known to the person skilled in the art (see e.g. http://en.wikipedia.org/wiki/Wobble_Hypothesis; Crick, J Mol Biol 19 (1966): 548-55).

In an alternative embodiment the present invention provides a nucleic acid sequence encoding an above described bispecific single chain antibody molecule of the invention.

The present invention also relates to a vector comprising the nucleic acid molecule of the present invention.

Many suitable vectors are known to those skilled in molecular biology, the choice of which would depend on the function desired and include plasmids, cosmids, viruses, bacteriophages and other vectors used conventionally in genetic engineering. Methods which are well known to those skilled in the art can be used to construct various plasmids and vectors; see, for example, the techniques described in

Sambrook et al. (loc cit.) and Ausubel, Current Protocols in Molecular Biology, Green Publishing Associates and Wiley Interscience, N.Y. (1989), (1994). Alternatively, the polynucleotides and vectors of the invention can be reconstituted into liposomes for delivery to target cells. As discussed in further details below, a cloning vector was used to isolate individual sequences of DNA. Relevant sequences can be transferred into expression vectors where expression of a particular polypeptide is required. Typical cloning vectors include pBluescript SK, pGEM, pUC9, pBR322 and pGBT9. Typical expression vectors include pTRE, pCAL-n-EK, pESP-1, pOP13CAT.

10 Preferably said vector comprises a nucleic acid sequence which is a regulatory sequence operably linked to said nucleic acid sequence defined herein.

The term "regulatory sequence" refers to DNA sequences, which are necessary to effect the expression of coding sequences to which they are ligated. The nature of such control sequences differs depending upon the host organism. In prokaryotes, control sequences generally include promoter, ribosomal binding site, and terminators. In eukaryotes generally control sequences include promoters, terminators and, in some instances, enhancers, transactivators or transcription factors. The term "control sequence" is intended to include, at a minimum, all components the presence of which are necessary for expression, and may also include additional advantageous components.

The term "operably linked" refers to a juxtaposition wherein the components so described are in a relationship permitting them to function in their intended manner. A control sequence "operably linked" to a coding sequence is ligated in such a way that expression of the coding sequence is achieved under conditions compatible with the control sequences. In case the control sequence is a promoter, it is obvious for a skilled person that double-stranded nucleic acid is preferably used.

Thus, the recited vector is preferably an expression vector. An "expression vector" is a construct that can be used to transform a selected host and provides for expression of a coding sequence in the selected host. Expression vectors can for instance be cloning vectors, binary vectors or integrating vectors. Expression comprises transcription of the nucleic acid molecule preferably into a translatable mRNA. Regulatory elements ensuring expression in prokaryotes and/or eukaryotic cells are well known to those skilled in the art. In the case of eukaryotic cells they comprise normally promoters ensuring initiation of transcription and optionally poly-A signals

ensuring termination of transcription and stabilization of the transcript. Possible regulatory elements permitting expression in prokaryotic host cells comprise, e.g., the P_L , *lac*, *trp* or *tac* promoter in *E. coli*, and examples of regulatory elements permitting expression in eukaryotic host cells are the *AOX1* or *GAL1* promoter in yeast or the
5 CMV-, SV40-, RSV-promoter (Rous sarcoma virus), CMV-enhancer, SV40-enhancer or a globin intron in mammalian and other animal cells.

Beside elements, which are responsible for the initiation of transcription such regulatory elements may also comprise transcription termination signals, such as the SV40-poly-A site or the tk-poly-A site, downstream of the polynucleotide.

10 Furthermore, depending on the expression system used leader sequences capable of directing the polypeptide to a cellular compartment or secreting it into the medium may be added to the coding sequence of the recited nucleic acid sequence and are well known in the art; see also the appended Examples. The leader sequence(s) is (are) assembled in appropriate phase with translation, initiation and termination
15 sequences, and preferably, a leader sequence capable of directing secretion of translated protein, or a portion thereof, into the periplasmic space or extracellular medium. Optionally, the heterologous sequence can encode a fusion protein including an N-terminal identification peptide imparting desired characteristics, e.g., stabilization or simplified purification of expressed recombinant product; see supra. In
20 this context, suitable expression vectors are known in the art such as Okayama-Berg cDNA expression vector pcDV1 (Pharmacia), pCDM8, pRc/CMV, pcDNA1, pcDNA3 (In-vitrogene), pEF-DHFR, pEF-ADA or pEF-neo (Mack et al. PNAS (1995) 92, 7021-7025 and Raum et al. Cancer Immunol Immunother (2001) 50(3), 141-150) or pSPORT1 (GIBCO BRL).

25 Preferably, the expression control sequences will be eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells, but control sequences for prokaryotic hosts may also be used. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences, and as desired, the
30 collection and purification of the bispecific single chain antibody molecule of the invention may follow; see, e.g., the appended examples.

An alternative expression system, which can be used to express a cell cycle interacting protein is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in

Spodoptera frugiperda cells or in *Trichoplusia* larvae. The coding sequence of a recited nucleic acid molecule may be cloned into a nonessential region of the virus, such as the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of said coding sequence will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein coat. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which the protein of the invention is expressed (Smith, J. Virol. 46 (1983), 584; Engelhard, Proc. Nat. Acad. Sci. USA 91 (1994), 3224-3227).

Additional regulatory elements may include transcriptional as well as translational enhancers. Advantageously, the above-described vectors of the invention comprise a selectable and/or scorable marker.

Selectable marker genes useful for the selection of transformed cells and, e.g., plant tissue and plants are well known to those skilled in the art and comprise, for example, antimetabolite resistance as the basis of selection for dhfr, which confers resistance to methotrexate (Reiss, Plant Physiol. (Life Sci. Adv.) 13 (1994), 143-149); npt, which confers resistance to the aminoglycosides neomycin, kanamycin and paromycin (Herrera-Estrella, EMBO J. 2 (1983), 987-995) and hygromycin (Marsh, Gene 32 (1984), 481-485). Additional selectable genes have been described, namely trpB, which allows cells to utilize indole in place of tryptophan; hisD, which allows cells to utilize histinol in place of histidine (Hartman, Proc. Natl. Acad. Sci. USA 85 (1988), 8047); mannose-6-phosphate isomerase which allows cells to utilize mannose (WO 94/20627) and ODC (ornithine decarboxylase) which confers resistance to the ornithine decarboxylase inhibitor, 2-(difluoromethyl)-DL-ornithine, DFMO (McConlogue, 1987, In: Current Communications in Molecular Biology, Cold Spring Harbor Laboratory ed.) or deaminase from *Aspergillus terreus* which confers resistance to Blasticidin S (Tamura, Biosci. Biotechnol. Biochem. 59 (1995), 2336-2338).

Useful scorable markers are also known to those skilled in the art and are commercially available. Advantageously, said marker is a gene encoding luciferase (Giacomin, Pl. Sci. 116 (1996), 59-72; Scikantha, J. Bact. 178 (1996), 121), green fluorescent protein (Gerdes, FEBS Lett. 389 (1996), 44-47) or β -glucuronidase (Jefferson, EMBO J. 6 (1987), 3901-3907). This embodiment is particularly useful for simple and rapid screening of cells, tissues and organisms containing a recited vector.

As described above, the recited nucleic acid molecule can be used alone or as part of a vector to express the bispecific single chain antibody molecule of the invention in cells, for, e.g., purification but also for gene therapy purposes. The nucleic acid molecules or vectors containing the DNA sequence(s) encoding any one of the

5 above described bispecific single chain antibody molecule of the invention is introduced into the cells which in turn produce the polypeptide of interest. Gene therapy, which is based on introducing therapeutic genes into cells by ex-vivo or in-vivo techniques is one of the most important applications of gene transfer. Suitable vectors, methods or gene-delivery systems for in-vitro or in-vivo gene therapy are

10 described in the literature and are known to the person skilled in the art; see, e.g., Giordano, *Nature Medicine* 2 (1996), 534-539; Schaper, *Circ. Res.* 79 (1996), 911-919; Anderson, *Science* 256 (1992), 808-813; Verma, *Nature* 389 (1994), 239; Isner, *Lancet* 348 (1996), 370-374; Muhlhauser, *Circ. Res.* 77 (1995), 1077-1086; Onodera, *Blood* 91 (1998), 30-36; Verma, *Gene Ther.* 5 (1998), 692-699; Nabel, *Ann. N.Y.*

15 *Acad. Sci.* 811 (1997), 289-292; Verzeletti, *Hum. Gene Ther.* 9 (1998), 2243-51; Wang, *Nature Medicine* 2 (1996), 714-716; WO 94/29469; WO 97/00957, US 5,580,859; US 5,589,466; or Schaper, *Current Opinion in Biotechnology* 7 (1996), 635-640; dos Santos Coura and Nardi *Virol J.* (2007), 4:99. The recited nucleic acid molecules and vectors may be designed for direct introduction or for introduction via

20 liposomes, or viral vectors (e.g., adenoviral, retroviral) into the cell. Preferably, said cell is a germ line cell, embryonic cell, or egg cell or derived there from, most preferably said cell is a stem cell. An example for an embryonic stem cell can be, inter alia, a stem cell as described in Nagy, *Proc. Natl. Acad. Sci. USA* 90 (1993), 8424-8428.

25

The invention also provides for a host transformed or transfected with a vector of the invention. Said host may be produced by introducing the above described vector of the invention or the above described nucleic acid molecule of the invention into the host. The presence of at least one vector or at least one nucleic acid molecule in the

30 host may mediate the expression of a gene encoding the above described single chain antibody constructs.

The described nucleic acid molecule or vector of the invention, which is introduced in the host may either integrate into the genome of the host or it may be maintained extrachromosomally.

The host can be any prokaryote or eukaryotic cell.

The term "prokaryote" is meant to include all bacteria, which can be transformed or transfected with DNA or RNA molecules for the expression of a protein of the invention. Prokaryotic hosts may include gram negative as well as gram positive
5 bacteria such as, for example, *E. coli*, *S. typhimurium*, *Serratia marcescens* and *Bacillus subtilis*. The term "eukaryotic" is meant to include yeast, higher plant, insect and preferably mammalian cells. Depending upon the host employed in a recombinant production procedure, the protein encoded by the polynucleotide of the present invention may be glycosylated or may be non-glycosylated. Especially
10 preferred is the use of a plasmid or a virus containing the coding sequence of the bispecific single chain antibody molecule of the invention and genetically fused thereto an N-terminal FLAG-tag and/or C-terminal His-tag. Preferably, the length of said FLAG-tag is about 4 to 8 amino acids, most preferably 8 amino acids. An above described polynucleotide can be used to transform or transfect the host using any of
15 the techniques commonly known to those of ordinary skill in the art. Furthermore, methods for preparing fused, operably linked genes and expressing them in, e.g., mammalian cells and bacteria are well-known in the art (Sambrook, loc cit.).

Preferably, said the host is a bacterium or an insect, fungal, plant or animal cell.

It is particularly envisaged that the recited host may be a mammalian cell. Particularly
20 preferred host cells comprise CHO cells, COS cells, myeloma cell lines like SP2/0 or NS/0. As illustrated in the appended examples, particularly preferred are CHO-cells as hosts.

More preferably said host cell is a human cell or human cell line, e.g. per.c6 (Kroos, Biotechnol. Prog., 2003, 19:163-168).

25 In a further embodiment, the present invention thus relates to a process for the production of a bispecific single chain antibody molecule of the invention, said process comprising culturing a host of the invention under conditions allowing the expression of the bispecific single chain antibody molecule of the invention and
30 recovering the produced polypeptide from the culture.

The transformed hosts can be grown in fermentors and cultured according to techniques known in the art to achieve optimal cell growth. The bispecific single chain antibody molecule of the invention can then be isolated from the growth medium, cellular lysates, or cellular membrane fractions. The isolation and

purification of the, e.g., microbially expressed bispecific single chain antibody molecules may be by any conventional means such as, for example, preparative chromatographic separations and immunological separations such as those involving the use of monoclonal or polyclonal antibodies directed, e.g., against a tag of the bispecific single chain antibody molecule of the invention or as described in the appended examples.

The conditions for the culturing of a host, which allow the expression are known in the art to depend on the host system and the expression system/vector used in such process. The parameters to be modified in order to achieve conditions allowing the expression of a recombinant polypeptide are known in the art. Thus, suitable conditions can be determined by the person skilled in the art in the absence of further inventive input.

Once expressed, the bispecific single chain antibody molecule of the invention can be purified according to standard procedures of the art, including ammonium sulfate precipitation, affinity columns, column chromatography, gel electrophoresis and the like; see, Scopes, "Protein Purification", Springer-Verlag, N.Y. (1982). Substantially pure polypeptides of at least about 90 to 95% homogeneity are preferred, and 98 to 99% or more homogeneity are most preferred, for pharmaceutical uses. Once purified, partially or to homogeneity as desired, the bispecific single chain antibody molecule of the invention may then be used therapeutically (including extracorporeally) or in developing and performing assay procedures. Furthermore, examples for methods for the recovery of the bispecific single chain antibody molecule of the invention from a culture are described in detail in the appended examples.

Furthermore, the invention provides for a composition comprising a bispecific single chain antibody molecule of the invention or a bispecific single chain antibody as produced by the process disclosed above. Preferably, said composition is a pharmaceutical composition.

The invention provides also for a bispecific single chain antibody molecule as defined herein, or produced according to the process as defined herein, wherein said bispecific single chain antibody molecule is for use in the prevention, treatment or amelioration of cancer. Preferably, said cancer is a solid tumor, more preferably a

carcinoma or prostate cancer. It is preferred that the bispecific single chain is further comprising suitable formulations of carriers, stabilizers and/or excipients. Moreover, it is preferred that said bispecific single chain antibody molecule is suitable to be administered in combination with an additional drug. Said drug may be a non-proteinaceous compound or a proteinaceous compound and may be administered
5 simultaneously or non-simultaneously with the bispecific single chain antibody molecule as defined herein.

In accordance with the invention, the term "pharmaceutical composition" relates to a
10 composition for administration to a patient, preferably a human patient. The particular preferred pharmaceutical composition of this invention comprises bispecific single chain antibodies directed against and generated against context-independent CD3 epitopes. Preferably, the pharmaceutical composition comprises suitable formulations of carriers, stabilizers and/or excipients. In a preferred embodiment, the
15 pharmaceutical composition comprises a composition for parenteral, transdermal, intraluminal, intraarterial, intrathecal and/or intranasal administration or by direct injection into tissue. It is in particular envisaged that said composition is administered to a patient via infusion or injection. Administration of the suitable compositions may be effected by different ways, e.g., by intravenous, intraperitoneal, subcutaneous,
20 intramuscular, topical or intradermal administration. In particular, the present invention provides for an uninterrupted administration of the suitable composition. As a non-limiting example, uninterrupted, i.e. continuous administration may be realized by a small pump system worn by the patient for metering the influx of therapeutic agent into the body of the patient. The pharmaceutical composition comprising the
25 bispecific single chain antibodies directed against and generated against context-independent CD3 epitopes of the invention can be administered by using said pump systems. Such pump systems are generally known in the art, and commonly rely on periodic exchange of cartridges containing the therapeutic agent to be infused. When exchanging the cartridge in such a pump system, a temporary interruption of the
30 otherwise uninterrupted flow of therapeutic agent into the body of the patient may ensue. In such a case, the phase of administration prior to cartridge replacement and the phase of administration following cartridge replacement would still be considered within the meaning of the pharmaceutical means and methods of the invention together make up one "uninterrupted administration" of such therapeutic agent.

The continuous or uninterrupted administration of these bispecific single chain antibodies directed against and generated against context-independent CD3 epitopes of this invention may be intravenous or subcutaneous by way of a fluid delivery device or small pump system including a fluid driving mechanism for driving fluid out of a reservoir and an actuating mechanism for actuating the driving mechanism. Pump systems for subcutaneous administration may include a needle or a cannula for penetrating the skin of a patient and delivering the suitable composition into the patient's body. Said pump systems may be directly fixed or attached to the skin of the patient independently of a vein, artery or blood vessel, thereby allowing a direct contact between the pump system and the skin of the patient. The pump system can be attached to the skin of the patient for 24 hours up to several days. The pump system may be of small size with a reservoir for small volumes. As a non-limiting example, the volume of the reservoir for the suitable pharmaceutical composition to be administered can be between 0.1 and 50 ml.

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

The composition of the present invention, comprising in particular bispecific single chain antibodies preferably directed against and generated against context-independent CD3 epitopes may further comprise a pharmaceutically acceptable carrier. Examples of suitable pharmaceutical carriers are well known in the art and include solutions, e.g. phosphate buffered saline solutions, water, emulsions, such as oil/water emulsions, various types of wetting agents, sterile solutions, liposomes, etc.

Compositions comprising such carriers can be formulated by well known conventional methods. Formulations can comprise carbohydrates, buffer solutions, amino acids and/or surfactants. Carbohydrates may be non-reducing sugars, preferably trehalose, sucrose, octasulfate, sorbitol or xylitol. Such formulations may be used for continuous administrations which may be intravenous or subcutaneous

with and/or without pump systems. Amino acids may be charged amino acids, preferably lysine, lysine acetate, arginine, glutamate and/or histidine. Surfactants may be detergents, preferably with a molecular weight of >1.2 KD and/or a polyether, preferably with a molecular weight of >3 KD. Non-limiting examples for preferred
5 detergents are TweenTM 20, Tween 40, Tween 60, Tween 80 or Tween 85. Non-limiting examples for preferred polyethers are PEG 3000, PEG 3350, PEG 4000 or PEG 5000. Buffer systems used in the present invention can have a preferred pH of 5-9 and may comprise citrate, succinate, phosphate, histidine and acetate. The compositions of the present invention can be administered to the subject at a suitable
10 dose which can be determined e.g. by dose escalating studies by administration of increasing doses of the bispecific single chain antibody molecule of the invention exhibiting cross-species specificity described herein to non-chimpanzee primates, for instance macaques. As set forth above, the bispecific single chain antibody molecule of the invention exhibiting cross-species specificity described herein can be
15 advantageously used in identical form in preclinical testing in non-chimpanzee primates and as drug in humans. These compositions can also be administered in combination with other proteinaceous and non-proteinaceous drugs. These drugs may be administered simultaneously with the composition comprising the bispecific single chain antibody molecule of the invention as defined herein or separately
20 before or after administration of said polypeptide in timely defined intervals and doses. The dosage regimen will be determined by the attending physician and clinical factors. As is well known in the medical arts, dosages for any one patient depend upon many factors, including the patient's size, body surface area, age, the particular compound to be administered, sex, time and route of administration, general health, and other drugs being administered concurrently. Preparations for
25 parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. Parenteral
30 vehicles include sodium chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers (such as those based on Ringer's dextrose), and the like. Preservatives and other additives may also be present such

as, for example, antimicrobials, anti-oxidants, chelating agents, inert gases and the like. In addition, the composition of the present invention might comprise proteinaceous carriers, like, e.g., serum albumin or immunoglobulin, preferably of human origin. It is envisaged that the composition of the invention might comprise, in addition to the bispecific single chain antibody molecule of the invention defined herein, further biologically active agents, depending on the intended use of the composition. Such agents might be drugs acting on the gastro-intestinal system, drugs acting as cytostatica, drugs preventing hyperurikemia, drugs inhibiting immunoreactions (e.g. corticosteroids), drugs modulating the inflammatory response, drugs acting on the circulatory system and/or agents such as cytokines known in the art.

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

chemistry parameters (e.g. lactate dehydrogenase) and other established standard methods may be used.

Another major challenge in the development of drugs such as the pharmaceutical composition of the invention is the predictable modulation of pharmacokinetic properties. To this end, a pharmacokinetic profile of the drug candidate, i.e. a profile of the pharmacokinetic parameters that effect the ability of a particular drug to treat a given condition, is established. Pharmacokinetic parameters of the drug influencing the ability of a drug for treating a certain disease entity include, but are not limited to:

half-life, volume of distribution, hepatic first-pass metabolism and the degree of blood serum binding. The efficacy of a given drug agent can be influenced by each of the parameters mentioned above.

"Half-life" means the time where 50% of an administered drug are eliminated through biological processes, e.g. metabolism, excretion, etc.

By "hepatic first-pass metabolism" is meant the propensity of a drug to be metabolized upon first contact with the liver, i.e. during its first pass through the liver.

"Volume of distribution" means the degree of retention of a drug throughout the various compartments of the body, like e.g. intracellular and extracellular spaces, tissues and organs, etc. and the distribution of the drug within these compartments.

"Degree of blood serum binding" means the propensity of a drug to interact with and bind to blood serum proteins, such as albumin, leading to a reduction or loss of biological activity of the drug.

Pharmacokinetic parameters also include bioavailability, lag time (Tlag), Tmax, absorption rates, more onset and/or Cmax for a given amount of drug administered.

"Bioavailability" means the amount of a drug in the blood compartment.

"Lag time" means the time delay between the administration of the drug and its detection and measurability in blood or plasma.

"Tmax" is the time after which maximal blood concentration of the drug is reached, and "Cmax" is the blood concentration maximally obtained with a given drug. The

time to reach a blood or tissue concentration of the drug which is required for its biological effect is influenced by all parameters. Pharmacokinetic parameters of bispecific single chain antibodies exhibiting cross-species specificity, which may be determined in preclinical animal testing in non-chimpanzee primates as outlined

above are also set forth e.g. in the publication by Schlereth et al. (Cancer Immunol. Immunother. 20 (2005), 1 – 12).

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

10 The term “safety”, “in vivo safety” or “tolerability” as used herein defines the administration of a drug without inducing severe adverse events directly after administration (local tolerance) and during a longer period of application of the drug. “Safety”, “in vivo safety” or “tolerability” can be evaluated e.g. at regular intervals during the treatment and follow-up period. Measurements include clinical evaluation, e.g. organ manifestations, and screening of laboratory abnormalities. Clinical
15 evaluation may be carried out and deviating to normal findings recorded/coded according to NCI-CTC and/or MedDRA standards. Organ manifestations may include criteria such as allergy/immunology, blood/bone marrow, cardiac arrhythmia, coagulation and the like, as set forth e.g. in the Common Terminology Criteria for adverse events v3.0 (CTCAE). Laboratory parameters which may be tested include
20 for instance haematology, clinical chemistry, coagulation profile and urine analysis and examination of other body fluids such as serum, plasma, lymphoid or spinal fluid, liquor and the like. Safety can thus be assessed e.g. by physical examination, imaging techniques (i.e. ultrasound, x-ray, CT scans, Magnetic Resonance Imaging (MRI), other measures with technical devices (i.e. electrocardiogram), vital signs, by
25 measuring laboratory parameters and recording adverse events. For example, adverse events in non-chimpanzee primates in the uses and methods according to the invention may be examined by histopathological and/or histochemical methods.

30 The term “effective and non-toxic dose” as used herein refers to a tolerable dose of the bispecific single chain antibody as defined herein which is high enough to cause depletion of pathologic cells, tumor elimination, tumor shrinkage or stabilization of disease without or essentially without major toxic effects. Such effective and non-toxic doses may be determined e.g. by dose escalation studies described in the art

and should be below the dose inducing severe adverse side events (dose limiting toxicity, DLT).

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

10 Moreover, the invention relates to a pharmaceutical composition comprising a bispecific single chain antibody molecule of this invention or produced according to the process according to the invention for the prevention, treatment or amelioration of cancer or an autoimmune disease. Preferably, said cancer is a:

- (a) a solid tumor, more preferably a carcinoma or prostate cancer;
- (b) a carcinoma, sarcoma, glioblastoma/astrocytoma, melanoma, mesothelioma, Wilms tumor or a hematopoietic malignancy such as leukemia, lymphoma or multiple myeloma;
- 15 (c) carcinomas (breast, kidney, lung, colorectal, colon, pancreas mesothelioma), sarcomas, and neuroectodermal tumors (melanoma, glioma, neuroblastoma);
- (d) epithelial cancer; or
- (e) bone or soft tissue cancer (e.g. Ewing sarcoma), breast, liver, lung, head and neck, colorectal, prostate, leiomyosarcoma, cervical and endometrial cancer,
- 20 ovarian, prostate, and pancreatic cancer.

Preferably, said pharmaceutical composition further comprises suitable formulations of carriers, stabilizers and/or excipients.

25 A further aspect of the invention relates to a use of a bispecific single chain antibody molecule/polypeptide as defined herein above or produced according to a process defined herein above, for the preparation of a pharmaceutical composition for the prevention, treatment or amelioration of a disease. Preferably, said disease is cancer. More preferably, said cancer is a solid tumor, preferably a carcinoma or prostate cancer.

30

In another preferred embodiment of use of the bispecific single chain antibody molecule of the invention said pharmaceutical composition is suitable to be administered in combination with an additional drug, i.e. as part of a co-therapy. In

said co-therapy, an active agent may be optionally included in the same pharmaceutical composition as the bispecific single chain antibody molecule of the invention, or may be included in a separate pharmaceutical composition. In this latter case, said separate pharmaceutical composition is suitable for administration prior to, simultaneously as or following administration of said pharmaceutical composition comprising the bispecific single chain antibody molecule of the invention. The additional drug or pharmaceutical composition may be a non-proteinaceous compound or a proteinaceous compound. In the case that the additional drug is a proteinaceous compound, it is advantageous that the proteinaceous compound be capable of providing an activation signal for immune effector cells.

Preferably, said proteinaceous compound or non-proteinaceous compound may be administered simultaneously or non-simultaneously with the bispecific single chain antibody molecule of the invention, a nucleic acid molecule as defined hereinabove, a vector as defined as defined hereinabove, or a host as defined as defined hereinabove.

Another aspect of the invention relates to a method for the prevention, treatment or amelioration of a disease in a subject in the need thereof, said method comprising the step of administration of an effective amount of a pharmaceutical composition of the invention. Preferably, said disease is cancer or an autoimmune disease. Preferably, said cancer is

- (a) a solid tumor, more preferably a carcinoma or prostate cancer;
- (b) a carcinoma, sarcoma, glioblastoma/astrocytoma, melanoma, mesothelioma, Wilms tumor or a hematopoietic malignancy such as leukemia, lymphoma or multiple myeloma;
- (c) carcinomas (breast, kidney, lung, colorectal, colon, pancreas mesothelioma), sarcomas, and neuroectodermal tumors (melanoma, glioma, neuroblastoma);
- (d) epithelial cancer; or
- (e) bone or soft tissue cancer (e.g. Ewing sarcoma), breast, liver, lung, head and neck, colorectal, prostate, leiomyosarcoma, cervical and endometrial cancer, ovarian, prostate, and pancreatic cancer.

In another preferred embodiment of the method of the invention said pharmaceutical composition is suitable to be administered in combination with an additional drug, i.e.

as part of a co-therapy. In said co-therapy, an active agent may be optionally included in the same pharmaceutical composition as the bispecific single chain antibody molecule of the invention, or may be included in a separate pharmaceutical composition. In this latter case, said separate pharmaceutical composition is suitable
5 for administration prior to, simultaneously as or following administration of said pharmaceutical composition comprising the bispecific single chain antibody molecule of the invention. The additional drug or pharmaceutical composition may be a non-proteinaceous compound or a proteinaceous compound. In the case that the additional drug is a proteinaceous compound, it is advantageous that the
10 proteinaceous compound be capable of providing an activation signal for immune effector cells.

Preferably, said proteinaceous compound or non-proteinaceous compound may be administered simultaneously or non-simultaneously with the bispecific single chain antibody molecule of the invention, a nucleic acid molecule as defined hereinabove,
15 a vector as defined as defined hereinabove, or a host as defined as defined hereinabove.

It is preferred for the above described method of the invention that said subject is a human.
20

In a further aspect, the invention relates to a kit comprising a bispecific single chain antibody molecule of the invention, a nucleic acid molecule of the invention, a vector of the invention, or a host of the invention.

25 These and other embodiments are disclosed and encompassed by the description and Examples of the present invention. Recombinant techniques and methods in immunology are described e.g. in Sambrook et al. Molecular Cloning: A Laboratory Manual; Cold Spring Harbor Laboratory Press, 3rd edition 2001; Lefkovits; Immunology Methods Manual; The Comprehensive Sourcebook of Techniques;
30 Academic Press, 1997; Golemis; Protein-Protein Interactions: A Molecular Cloning Manual; Cold Spring Laboratory Press, 2002. Further literature concerning any one of the antibodies, methods, uses and compounds to be employed in accordance with the present invention may be retrieved from public libraries and databases, using for example electronic devices. For example, the public database "Medline", available on

the Internet, may be utilized, for example under <http://www.ncbi.nlm.nih.gov/PubMed/medline.html>. Further databases and addresses such as <http://www.ncbi.nlm.nih.gov/> or listed at the EMBL-services homepage under <http://www.embl.de/services/index.html> are known to the person skilled in the art and can also be obtained using, e. g., <http://www.google.com>.

The figures show:

Figure 1

Flowcytometry of CHO cells transfected with native human EpCAM (positive control) and different EpCAM-hNG2 fusion proteins, respectively. Staining with the murine parental IgG1 antibody MAb 5-10 (bold lines) directed against human EpCAM was performed as described (Brischwein (2007) J Immunother 30: 798-807). PBS/2%FCS instead of MAb 5-10 was used as negative control (thin lines).

Figure 2

T cell cytotoxicity redirected by bscAb 5-10 x I2C against CHO cells transfected with human EpCAM (positive control) and different EpCAM-hNG2 fusion proteins as measured in a chromium 51 (^{51}Cr) release assay (y-axis). Stimulated human CD4/CD56 depleted PBMC served as effector T cells. Effector- to target cell ratio was 10:1. BscAb 5-10 x I2C was used as culture supernatant at different dilutions as indicated on the x-axis. Assay duration was 18 hours.

Figure 3

Amino acid sequence alignment of full-length mature human and rat PSMA. Mismatching homologous amino acid positions are underlined and highlighted by bold character style. Numbering of amino acid positions refers to human PSMA and starts with the amino acid methionine encoded by the start codon of human PSMA. Intracellular domain aa 1 - aa 19; transmembrane domain aa 20 - aa 43; extracellular domain aa 44 – aa 750.

Figure 4

FACS binding analysis of I2C-based anti-human PSMA-bscAbs on CHO cells transfected with (unmutated) human PSMA, rat PSMA mutated to the homologous human amino acid at every mismatched amino acid position with a membrane-distance of $\geq 60\text{\AA}$, and unmutated rat PSMA. The bold lines show staining by cell culture supernatant of CHO cells transfected with PSMA-directed bispecific antibody constructs. Cell culture supernatant of untransfected CHO cells served as negative

control (thin lines). PSMA-directed bscAbs P1xI2C, P2xI2C, P3xI2C, P4xI2C and P5xI2C bind to (unmutated) human PSMA but neither to unmutated nor to mutated rat PSMA and thus confirms a membrane-distance of $< 60\text{\AA}$ for the PSMA-epitope of each of these bispecific constructs. By contrast, PSMA-directed bscAbs D1xI2C and D2xI2C do not bind to unmutated rat PSMA but to (unmutated) human and mutated rat PSMA consistent with PSMA-epitopes of a membrane-distance $\geq 60\text{\AA}$.

Figure 5

T cell cytotoxicity redirected by I2C-based PSMA-directed bscAbs to CHO cells transfected with human PSMA as measured in a chromium 51 (^{51}Cr) release assay. As source of effector T cells stimulated human CD4/CD56 depleted PBMC were used. The effector-to-target cell ratio was 10:1. PSMA-directed bscAbs were used as cell culture supernatants from transfected CHO cells at different dilutions as indicated. The assay duration was 18 hours. PSMA-directed bscAbs P1xI2C, P2xI2C, P3xI2C, P4xI2C and P5xI2C, whose PSMA-epitopes have a membrane-distance of $< 60\text{\AA}$ are substantially more potent in redirecting T cell cytotoxicity than PSMA-directed bscAbs D1xI2C and D2xI2C, whose PSMA-epitopes have a membrane-distance of $\geq 60\text{\AA}$.

Figure 6

FACS binding analysis of I2C-based anti-human PSMA-bscAbs on CHO cells transfected with (unmutated) human PSMA, rat PSMA mutated to the homologous human amino acid at every mismatched amino acid position with a membrane-distance of $\geq 60\text{\AA}$, and unmutated rat PSMA. The bold lines show staining by cell culture supernatant of CHO cells transfected with PSMA-directed bispecific antibody constructs. Cell culture supernatant of untransfected CHO cells served as negative control (thin lines). The PSMA-directed bscAb P6xI2C binds to (unmutated) human PSMA but neither to unmutated nor to mutated rat PSMA and thus confirms a membrane-distance of $< 60\text{\AA}$ for the PSMA-epitope of this bispecific construct. By contrast, PSMA-directed bscAb D3xI2C does not bind to unmutated rat PSMA but to (unmutated) human and mutated rat PSMA consistent with a PSMA-epitope of a membrane-distance $\geq 60\text{\AA}$.

Figure 7

T cell cytotoxicity redirected by I2C-based PSMA-directed bscAbs to CHO cells transfected with human PSMA as measured in a chromium 51 (^{51}Cr) release assay. As source of effector T cells stimulated human CD4/CD56 depleted PBMC were

used. The effector-to-target cell ratio was 10:1. PSMA-directed bscAbs were used as cell culture supernatants from transfected CHO cells at different dilutions as indicated. The assay duration was 18 hours. PSMA-directed bscAb P6xI2C, whose PSMA-epitope has a membrane-distance of $< 60\text{\AA}$ is substantially more potent in
5 redirecting T cell cytotoxicity than PSMA-directed bscAb D3xI2C, whose PSMA-epitope has a membrane-distance of $\geq 60\text{\AA}$.

Figure 8

FACS binding analysis of I2C-based bscAbs directed to membrane-proximal PSMA-epitopes on CHO cells transfected with macaque PSMA, the CD3 positive human T
10 cell leukemia cell line HPB-ALL and the CD3 positive macaque T cell line 4119LnPx. The bold lines show staining by cell culture supernatant of CHO cells transfected with designated PSMA-directed bispecific antibody constructs. Cell culture supernatant of untransfected CHO cells served as negative control (thin lines). The designated PSMA-directed bscAbs in addition to human PSMA also bind to macaque PSMA,
15 human CD3 and macaque CD3.

Figure 9

Amino acid sequence alignment of full-length mature human and murine FAP α . Mismatching homologous amino acid positions are underlined and highlighted by bold character style. Numbering of amino acid positions refers to human FAP α and starts with the amino acid methionine encoded by the start codon of human
20 FAP α . Intracellular domain aa 1 - aa 9; transmembrane domain aa 10 - aa 26; extracellular domain aa 27 – aa 760.

Figure 10

FACS binding analysis of cell surface expression on CHO cells expressing the
25 murine FAP α antigen as described in Example 6.3 and CHO cells expressing the mutated human FAP α antigen with murine membrane-distal epitopes as described in Example 6.4, respectively. The FACS staining was performed as described in Examples 6.3 and 6.4. The bold lines represent cells incubated with the detection antibodies – the Penta His antibody in case of murine FAP α and the
30 anti-FLAG M2 antibody in case of the mutated human FAP α antigen with murine membrane-distal epitopes. The thin lines represent the negative controls. For both cell lines the overlay of the histograms for the anti-FLAG M2 antibody and the Penta His antibody, respectively, shows a significant expression level of the respective antigen. Expression levels were comparable for the two cell lines.

Figure 11

FACS binding analysis of designated bispecific single chain constructs to CHO cells expressing human FAPalpha as described in Example 6.1, the human CD3+ T cell line HPB-ALL, CHO cells expressing macaque FAPalpha as described in Example 6.15 and the macaque T cell line 4119LnPx, respectively. The FACS staining was performed as described in Examples 6.13 and 6.16. The bold lines represent cells incubated with cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The thin lines represent the negative controls. Supernatant of untransfected CHO cells was used as negative control. For each bispecific single chain construct the overlays of the histograms show specific binding of the construct to human and macaque FAPalpha and human and macaque CD3.

Figure 12

The diagrams show results of chromium release assays measuring cytotoxic activity induced by designated FAPalpha specific single chain constructs redirected to the indicated target cell lines generated as described in Examples 6.1, 6.3 and 6.4. Effector cells were also used as indicated. The assays were performed as described in Example 6.14. The diagrams clearly demonstrate for each construct the potent recruitment of cytotoxic activity of human effector T cells against target cells positive for human FAPalpha and target cells positive for the mutated human FAPalpha antigen with murine membrane-distal epitopes. No significant recruitment of cytotoxic activity of human effector T cells against target cells positive for murine FAPalpha was detectable.

Figure 13

FACS binding analysis of designated bispecific single chain constructs to CHO cells expressing human c-MET as described in Example 7.17, the human CD3+ T cell line HPB-ALL, CHO cells expressing macaque c-MET as described in Example 7.17 and the macaque T cell line 4119LnPx, respectively. The FACS staining was performed as described in Examples 7.13 and 7.16. The bold lines represent cells incubated with cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The filled histograms show the negative controls. Supernatant of untransfected CHO cells was used as negative control. For each bispecific single chain construct the overlays of the histograms show specific binding of the construct to human and macaque c-MET and human and macaque CD3.

Figure 14

FACS binding analysis of designated bispecific single chain constructs to CHO cells expressing the murine c-MET antigen as described in Example 7.17, CHO cells expressing the mutated human c-MET antigen with murine membrane-distal epitopes as described in Example 7.17 and CHO cells expressing the mutated murine c-MET antigen with human membrane-distal epitopes as described in Example 7.17, respectively. The FACS staining was performed as described in Example 7.13. The bold lines represent cells incubated with cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The filled histograms show the negative controls. Supernatant of untransfected CHO cells was used as negative control. An anti-FLAG M2 antibody was used to detect expression levels of the respective antigens. For each cell line the overlay of the histograms for the anti-FLAG M2 antibody shows high expression levels of the respective antigen. Expression levels were comparable for the three cell lines. For each bispecific single chain construct the overlays of the histograms show specific binding of the construct to the mutated human c-MET antigen with murine membrane-distal epitopes but not to the mutated murine c-MET antigen with human membrane-distal epitopes and not to the murine c-MET antigen.

Figure 15

The diagrams show results of chromium release assays measuring cytotoxic activity induced by designated c-MET specific single chain constructs redirected to the indicated target cell lines generated as described in Example 7.17. Effector cells were also used as indicated. The assays were performed as described in Example 7.14. The diagrams clearly demonstrate for each construct the potent recruitment of cytotoxic activity of human effector T cells against target cells positive for human c-MET. No significant recruitment of cytotoxic activity of human effector T cells against target cells positive for murine c-MET and target cells positive for the mutated murine c-MET antigen with human membrane-distal epitopes, respectively, was detectable.

Figure 16

FACS binding analysis of designated cross-species specific scFv antibodies to CHO cells expressing human c-MET as described in Example 7.17, CHO cells expressing the murine c-MET antigen as described in Example 7.17, CHO cells expressing the mutated human c-MET antigen with murine membrane-distal epitopes as described in Example 7.17 and CHO cells expressing the mutated murine c-MET antigen with human membrane-distal epitopes as described in Example 7.17, respectively. The

FACS staining was performed as described in Example 7.9. The bold lines represent cells incubated with periplasmic preparations containing the c-MET specific scFv antibodies. The filled histograms show the negative controls. The Buffer used for periplasmic preparations was used as negative control. For each c-MET specific scFv antibody the overlays of the histograms show specific binding of the construct to human c-MET and human c-MET with murine membrane-distal epitopes. No significant binding to cells positive for murine c-MET and to cells positive for the mutated murine c-MET with human membrane-distal epitopes, respectively, was detectable.

Figure 17

FACS binding analysis of designated bispecific single chain constructs to CHO cells expressing human IGF-1R as described in Example 9.1, the human CD3+ T cell line HPB-ALL, CHO cells expressing macaque IGF-1R as described in Example 9.15 and the macaque T cell line 4119LnPx, respectively. The FACS staining was performed as described in Examples 9.13 and 9.16. The bold lines represent cells incubated with cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The filled histograms show the negative controls. Supernatant of untransfected CHO cells was used as negative control. For each bispecific single chain construct the overlays of the histograms show specific binding of the construct to human and macaque IGF-1R and human and macaque CD3.

Figure 18

FACS binding analysis of designated bispecific single chain constructs to CHO cells expressing the murine IGF-1R antigen as described in Example 9.3, CHO cells expressing the mutated human IGF-1R antigen with murine membrane-distal epitopes as described in Example 9.4 and CHO cells expressing the mutated murine IGF-1R antigen with human membrane-distal epitopes as described in Example 9.5, respectively. The FACS staining was performed as described in Example 9.13. The bold lines represent cells incubated with cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The filled histograms show the negative controls. Supernatant of untransfected CHO cells was used as negative control. For each bispecific single chain construct the overlays of the histograms show specific binding of the construct to the mutated human IGF-1R antigen with murine membrane-distal epitopes but not to the mutated murine IGF-1R antigen with human membrane-distal epitopes and not to the murine IGF-1R antigen.

Figure 19

The diagrams show results of chromium release assays measuring cytotoxic activity induced by designated IGF-1R specific single chain constructs redirected to the CHO cells expressing human IGF-1R as described in Example 9.1. Effector cells were used as indicated. The assays were performed as described in Example 9.14. The diagrams clearly demonstrate for each construct the potent recruitment of cytotoxic activity of human effector T cells against target cells positive for human IGF-1R.

Figure 20

FACS binding analysis of designated bispecific single chain constructs to CHO cells expressing designated human / rat PSMA chimeras as described in Example 10.2.1. The FACS staining was performed as described in Example 10.2.2. The bold lines represent cells incubated with cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The filled histograms show the negative controls. Supernatant of untransfected CHO cells was used as negative control. For each bispecific single chain construct the overlays of the histograms show specific binding of the construct to the chimeric constructs huPSMArat140-169, huPSMArat281-284, huPSMArat300-344, huPSMArat683-690 and huPSMArat716-750. Compared with the signals obtained for the other bispecific single chain construct there is a clear lack of binding for the bispecific single chain antibody construct PSMA-P7 HL x I2C HL to the chimeric PSMA construct huPSMArat598-617.

Figure 21

The Figure shows binding signals obtained with periplasmic preparations of the scFv antibody of the PSMA specific binder of PSMA-D4 HL x I2C HL to 15-mer peptides spanning over the extracellular domain of human PSMA and overlapping with their neighboring peptides by 14 amino acids. Signals obtained for the peptides are plotted on the X-axis in order of the N-terminal peptides on the left to the C-terminal peptides on the right. Strength of ELISA signals using His detection is plotted on the Y-axis. The ELISA was performed as described in Example 10.3. A distinct maximum signal is detectable for the peptide spanning over the amino acids threonine 334 to threonine 339.

Figure 22

The diagram shows results of a CytoTox-Glo™ cytotoxicity assay measuring cytotoxic activity of unstimulated human T cells induced by designated PSMA specific bispecific single chain constructs against CHO cells expressing human

PSMA as described in Example 2.1. The assay was performed as described in Example X.4. The diagram clearly demonstrates the superior cytotoxic activity of PSMA bispecific single chain antibody PSMA-P7 HL x I2C HL directed at a membrane-proximal target epitope of human PSMA over PSMA bispecific single chain antibody PSMA-D4 HL x I2C HL directed at a membrane-distal target epitope of human PSMA.

Table legends

Table 1

All extracellular amino acids of human PSMA mismatching with the homologous rat PSMA amino acid sequence. Those mismatched extracellular human PSMA amino acids, whose alpha C-atoms have a distance of ≥ 60 Å from the alpha C-atom of the thirteenth extracellular human PSMA amino acid (i.e. the reference aa) as counted from the junction of transmembrane and extracellular region are marked in bold. The distances between alpha C-atoms of two amino acids within human PSMA were determined using the crystal structure of human PSMA (accession No 1Z8L; obtained from the RCSB pdb, protein data bank of the Research Collaboratory for Structural Bioinformatics; <http://www.rcsb.org/pdb>) and the "measure distance mode" of the software "3D molecule viewer" (a component of Vector NTI Suite 8.0, Informax Inc.). Numbering of amino acid positions refers to human PSMA and starts with the amino acid methionine encoded by the start codon of human PSMA. The reference aa is histidine at position 56.

Table 2

All extracellular amino acids of human FAPalpha mismatching with the homologous murine FAPalpha amino acid sequence. Those mismatched extracellular human FAPalpha amino acids, whose alpha C-atoms have a distance of ≥ 60 Å from the alpha C-atom of the thirteenth extracellular human FAPalpha amino acid (i.e. the reference aa) as counted from the junction of transmembrane and extracellular region are marked in bold. The distances between alpha C-atoms of two amino acids within human FAPalpha were determined using the crystal structure of human FAPalpha (Accession No 1Z68; obtained from the RCSB pdb, protein data bank of the Research Collaboratory for Structural Bioinformatics; <http://www.rcsb.org/pdb>) and the "measure distance mode" of the software "3D molecule viewer" (a component of Vector NTI Suite 8.0, Informax Inc.). Numbering of amino acid

positions refers to human FAPalpha and starts with the amino acid methionine encoded by the start codon of human FAPalpha. The reference aa is methionine at position 39.

- 5 The present invention is additionally described by way of the following illustrative non-limiting examples that provide a better understanding of the present invention and of its many advantages.

EXAMPLES

10 **1. Cytotoxicity with respect to the distance of the target cell epitopes distance from the target cell membrane**

1.1. Generation of CHO cells expressing the human EpCAM antigen

- The sequence of the human EpCAM antigen ('NM_002354, Homo sapiens tumor-associated calcium signal transducer 1 (TACSTD1), mRNA, National Center for
15 Biotechnology Information, <http://www.ncbi.nlm.nih.gov/entrez>) was used to obtain a synthetic molecule by gene synthesis according to standard protocols. The gene synthesis fragment was also designed as to contain a Kozak site for eukaryotic expression of the construct and and restriction sites at the beginning and the end of the DNA. The introduced restriction sites XbaI at the 5' end and Sall at the 3' end
20 were utilised in the following cloning procedures. The gene synthesis fragment was cloned via XbaI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures were carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring
25 Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence was transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct was induced by
30 increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

1.2. Generation of CHO cells expressing EpCAM-hNG2 fusion proteins

The coding sequences of EpCAM-hNG2 fusion proteins EpCAM-D1-hNG2 (SEQ ID Nos 189 and 190), EpCAM-D3-hNG2 (SEQ ID Nos 191 and 192), EpCAM-D1D3-hNG2 (SEQ ID Nos 193 and 194), EpCAM-D1D2-hNG2 (SEQ ID Nos 195 and 196) and EpCAM-hNG2 (SEQ ID Nos 197 and 198) were obtained by gene synthesis according to standard protocols. The gene synthesis fragment was designed as to contain first the coding sequence of an immunoglobulin leader peptide followed subsequently by human EpCAM, the respective extracellular part of human NG2 and the transmembrane and cytoplasmic domain of the human NG2 (Pluschke (1996) PNAS 93: 9710-9715). The different components were connected by short peptide linkers. The gene synthesis fragments were also designed as to introduce restriction sites at the 5' end (Eco RI) and at the 3' end (Sal I) for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures were carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). For each EpCAM-hNG2 fusion protein a clone with sequence-verified nucleotide sequence was transfected into DHFR deficient CHO cells for eukaryotic expression. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct was induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

1.3. Generation of the EpCAM and CD3 bispecific single antibody 5-10 x I2C

The bispecific single chain antibody 5-10 x I2C comprising the scFv binding domain 5-10 (in VL-VH arrangement) directed at human EpCAM (Brischwein (2007) J Immunother 30: 798-807) and the scFv binding domain I2C (in VL-VH arrangement) directed at CD3epsilon on human T cells was obtained by gene synthesis. The gene synthesis fragment was designed as to contain first a Kozak site for eukaryotic expression of the construct, followed by a 19 amino acid immunoglobulin leader peptide, followed in frame by the coding sequence of the bispecific single chain antibody 5-10 x I2C, followed in frame by the coding sequence of a 6 histidine tag and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 199 and 200). The gene synthesis fragment was also designed as to introduce suitable restriction sites at the beginning (EcoRI) and at the end of the

fragment (Sal I) for cloning of the gene synthesis fragment into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures were carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence was transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct was induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. After two passages of stationary culture cell culture supernatant was collected and used in the subsequent experiments.

1.4. Flowcytometry of CHO cells transfected with different EpCAM-hNG2 fusion proteins

The presence of the EpCAM-epitope of bispecific single chain antibody 5-10 x I2C on the CHO cells transfected with native EpCAM and the EpCAM-hNG2 fusion proteins EpCAM-D1-hNG2, EpCAM-D3-hNG2, EpCAM-D1D3-hNG2, EpCAM-D1D2-hNG2 and EpCAM-hNG2, respectively, was confirmed by flowcytometry with the murine parental IgG1 antibody Mab 5-10 as described (Brischwein (2007) J Immunother 30: 798-807). The result is shown in Figure 1.

1.5. T cell cytotoxicity redirected by bscAb 5-10 x I2C against CHO cells transfected with different EpCAM-hNG2 fusion proteins

T cell cytotoxicity redirected by bscAb 5-10 x I2C against CHO cells transfected with different EpCAM-hNG2 fusion proteins was measured in a chromium 51 (^{51}Cr) release in vitro cytotoxicity assay. As source of effector T cells stimulated human CD4/CD56 depleted PBMC were used. Stimulated human PBMC were obtained as follows: A Petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) was coated with a commercially available anti-CD3 specific antibody (e.g. OKT3, Orthoclone) in a final concentration of 1 $\mu\text{g}/\text{ml}$ for 1 hour at 37°C. Unbound protein was removed by one washing step with PBS. The fresh PBMC were isolated from peripheral blood (30 – 50 ml human blood) by FicollTM gradient centrifugation according to standard protocols. 3 - 5 x 10⁷ PBMC were added to the precoated petri dish in

120 ml of RPMI 1640 with stabilized glutamine / 10% FCS / IL-2 20 U/ml (Proleukin, Chiron) and stimulated for 2 days. On the third day the cells were collected and washed once with RPMI 1640. IL-2 was added to a final concentration of 20 U/ml and the cells were cultured again for one day in the same cell culture medium as above.

- 5 By depletion of CD4⁺ T cells and CD56⁺ NK cells according to standard protocols CD8⁺ cytotoxic T lymphocytes (CTLs) were enriched. Target cells were washed twice with PBS and labelled with 11.1 MBq ⁵¹Cr in a final volume of 100µl RPMI with 50% FCS for 60 minutes at 37°C. Subsequently the labelled target cells were washed 3 times with 5 ml RPMI and then used in the cytotoxicity assay. The assay
- 10 was performed in a 96 well plate in a total volume of 250µl supplemented RPMI (as above) with an E:T ratio of 10:1. BscAb 5-10 x I2C was added as culture supernatant from transfected CHO cells at different dilutions. The assay time was 18 hours. Cytotoxicity was measured as relative values of released chromium related to the difference of maximum lysis (addition of Triton-XTM) and spontaneous lysis (without
- 15 effector cells). All measurements were carried out in quadruplicates. Measurement of released chromium activity was performed with a Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany). Analysis of the experimental data was performed with Prism 4 for Windows (version 4.02, GraphPad Software Inc., San Diego, California, USA). Sigmoidal dose response curves typically had R² values
- 20 >0.90 as determined by the software. As shown in Figure 2 the T cell cytotoxicity redirected by bscAb 5-10 x I2C against the indicated target cells critically depends on the varying distance of the target EpCAM epitope of bsc 5-10 x I2C in the different EpCAM-hNG2 model antigens from the target cell membrane. The descending order of T cell cytotoxicity with increasing membrane distance of the target epitope as
- 25 given in parentheses is EpCAM-CHO (positive control) > EpCAM-D1-hNG2 (640 aa) > EpCAM-D3-hNG2 (679 aa) > EpCAM-D1D3-hNG2 (871 aa). There was no cytotoxic activity detectable against CHO cells expressing EpCAM-D1D2-hNG2 (1511 aa) or EpCAM-hNG2 (2190 aa).

30 **2. Generation of bispecific single chain antibodies directed at membrane-proximal target epitopes of human PSMA**

2.1 Generation of CHO cells expressing human PSMA

The coding sequence of human PSMA as published in GenBank (Accession number NM_004476) is obtained by gene synthesis according to standard protocols. The

gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the human PSMA protein and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 201 and 202). The gene synthesis fragment is also
5 designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, XbaI at the 5' end and SalI at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via XbaI and SalI into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following
10 standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in
15 DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

2.2 Generation of a soluble human PSMA fusion protein

20 The coding sequence of human PSMA as described in Example 2.1 and the coding sequence of murine Lag3 as published in GenBank (Accession number NM_008479) are used for the construction of an artificial cDNA sequence encoding a soluble fusion protein of human PSMA and murine Lag3. To generate a construct for expression of the soluble human PSMA fusion protein a cDNA fragment is obtained
25 by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 203 and 204). The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the murine Lag3 protein from amino acid 1 to 441 corresponding to the signal peptide and
30 extracellular domains of murine Lag3, followed in frame by the coding sequence of an artificial Ser₁-Gly₄-Ser₁-linker, followed in frame by the coding sequence of the human PSMA protein from amino acid 44 to 750 corresponding to the extracellular domains of human PSMA, followed in frame by the coding sequence of an artificial Ser₁-Gly₁-linker, followed in frame by the coding sequence of a 6 histidine tag and a

stop codon. The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, XbaI at the 5' end and SalI at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via XbaI and SalI into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are all carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. After two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F – 68; HyCloneTM) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively a clone of the expression plasmid with sequence-verified nucleotide sequence is used for transfection and protein expression in the FreeStyle 293 Expression System (Invitrogen GmbH, Karlsruhe, Germany) according to the manufacturer's protocol. Supernatant containing the expressed protein is obtained, cells are removed by centrifugation and the supernatant is stored at -20°C.

Purification of the soluble human PSMA fusion protein is performed as follows: Äkta® Explorer System (GE Health Systems) and Unicorn® Software are used for chromatography. Immobilized metal affinity chromatography ("IMAC") is performed using a Fractogel EMD chelate® (Merck) which is loaded with ZnCl₂ according to the protocol provided by the manufacturer. The column is equilibrated with buffer A (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl) and the cell culture supernatant (500 ml) is applied to the column (10 ml) at a flow rate of 3 ml/min. The column is washed with buffer A to remove unbound sample. Bound protein is eluted using a two step gradient of buffer B (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl, 0.5 M Imidazole) according to the following procedure:

Step 1: 20% buffer B in 6 column volumes

Step 2: 100% buffer B in 6 column volumes

Eluted protein fractions from step 2 are pooled for further purification. All chemicals are of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

- 5 Gel filtration chromatography is performed on a HiLoadTM 16/60 Superdex 200 prep grade column (GE/Amersham) equilibrated with Equi-buffer (25 mM Citrate, 200 mM Lysine, 5% Glycerol, pH 7.2). Eluted protein samples (flow rate 1 ml/min) are subjected to standard SDS-PAGE and Western Blot for detection. Prior to purification, the column is calibrated for molecular weight determination (molecular weight marker kit, Sigma MW GF-200). Protein concentrations are determined using OD280 nm.

2.3 Generation of CHO cells expressing rat PSMA

- The sequence of rat PSMA (NM_057185, *Rattus norvegicus* folate hydrolase (Folh1), mRNA, National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/entrez>) is used to obtain a synthetic cDNA molecule by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the complete coding sequence of the rat PSMA antigen, followed in frame by the coding sequence of a FLAG-tag and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 205 and 206). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sall) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

2.4 Generation of CHO cells expressing a mutated human PSMA antigen with rat membrane-distal epitopes

The coding sequence of a mutated human PSMA antigen with rat membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the complete coding sequence of the human PSMA antigen mutated at 12 specific amino acid positions as explained below, followed in frame by the coding sequence of a FLAG tag and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 207 and 208).

All extracellular amino acids of human PSMA mismatching with the homologous rat sequence, whose alpha C-atoms have a distance of ≥ 60 Å from the alpha C-atom of the thirteenth extracellular amino acid (i.e. the reference aa) as counted from the junction of transmembrane and extracellular region, are mutated to the homologous mismatched rat amino acid. This applies to the 12 amino acids that are listed in table Table 1 and marked in bold. The homologous mismatched amino acids between human and rat PSMA are identified by sequence alignment as shown in Figure 3. The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (Xba I) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

2.5 Generation of CHO cells expressing a mutated rat PSMA antigen with human membrane-distal epitopes

The coding sequence of a mutated rat PSMA antigen with human membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the complete coding sequence of the rat

PSMA antigen mutated at the same 12 specific extracellular amino acid positions as identified in the foregoing Example 2.4 to the respective homologous human amino acid, followed in frame by the coding sequence of a FLAG tag and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 209 and 210). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI I) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566.

Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

2.6 Immunization of mice using a soluble human PSMA fusion protein

Twelve weeks old F1 mice from BALB/c x C57BL/6 crossings are immunized with the soluble human PSMA fusion protein as described in Example 2.2 To this end for each animal 40 µg of the soluble human PSMA fusion protein are mixed with 10 nmol of a thioate-modified CpG-Oligonucleotide (5'-tccatgacgttctctgatgct-3') in 300 µl PBS and are injected intraperitoneally. Mice receive booster immunizations after 21, 42 and optionally 63 days in the same way. Ten days after the first booster immunization, blood samples are taken and antibody serum titers against human PSMA are tested by flow cytometry according to standard protocols. To this end 200.000 cells of the human PSMA transfected CHO cells as described in Example 2.1 are incubated for 30 min on ice with 50 µl of serum of the immunized animals diluted 1:1000 in PBS with 2% FCS. The cells are washed twice in PBS with 2% FCS and binding of serum antibodies is detected with an mouse Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Serum of the animals obtained prior to immunization is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as

described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Animals demonstrating significant serum reactivity against human PSMA as determined by the FACS analysis are used in the subsequent experiment.

5 **2.7 Generation of an immune murine antibody scFv library: Construction of a combinatorial antibody library and phage display**

Three days after the last booster immunization spleen cells of reactive animals are harvested for the preparation of total RNA according to standard protocols.

A library of murine immunoglobulin (Ig) light chain (kappa) variable region (VK) and
10 Ig heavy chain variable region (VH) DNA-fragments is constructed by RT-PCR on murine spleen RNA using VK-and VH specific primers. cDNA is synthesized according to standard protocols.

The primers are designed in a way to give rise to a 5'-XhoI and a 3'-BstEI recognition site for the amplified heavy chain V-fragments and to a 5'-SacI and a 3'-SpeI
15 recognition site for amplified VK DNA fragments.

For the PCR-amplification of the VH DNA-fragments eight different 5'-VH-family specific primers (MVH1(GC)AG GTG CAG CTC GAG GAG TCA GGA CCT SEQ ID NO: 211; MVH2 GAG GTC CAG CTC GAG CAG TCT GGA CCT SEQ ID NO: 212; MVH3 CAG GTC CAA CTC GAG CAG CCT GGG GCT SEQ ID NO: 213; MVH4
20 GAG GTT CAG CTC GAG CAG TCT GGG GCA SEQ ID NO: 214; MVH5 GA(AG) GTG AAG CTC GAG GAG TCT GGA GGA SEQ ID NO: 215; MVH6 GAG GTG AAG CTT CTC GAG TCT GGA GGT SEQ ID NO: 216; MVH7 GAA GTG AAG CTC GAG GAG TCT GGG GGA SEQ ID NO: 217; MVH8 GAG GTT CAG CTC GAG CAG TCT GGA GCT SEQ ID NO: 218) are each combined with one 3'-VH primer
25 (3'MuVHBstEI tga gga gac ggt gac cgt ggt ccc ttg gcc cca g SEQ ID NO: 219); for the PCR amplification of the VK-chain fragments seven different 5'-VK-family specific primers (MUVK1 CCA GTT CCG AGC TCG TTG TGA CTC AGG AAT CT SEQ ID NO: 220; MUVK2 CCA GTT CCG AGC TCG TGT TGA CGC AGC CGC CC SEQ ID NO: 221; MUVK3 CCA GTT CCG AGC TCG TGC TCA CCC AGT CTC CA SEQ ID
30 NO: 222; MUVK4 CCA GTT CCG AGC TCC AGA TGA CCC AGT CTC CA SEQ ID NO: 223; MUVK5 CCA GAT GTG AGC TCG TGA TGA CCC AGA CTC CA SEQ ID NO: 224; MUVK6 CCA GAT GTG AGC TCG TCA TGA CCC AGT CTC CA SEQ ID NO: 225; MUVK7 CCA GTT CCG AGC TCG TGA TGA CAC AGT CTC CA SEQ ID

NO: 226) are each combined with one 3'-VK primer (3'MuVkJHindIII/BsiW1 tgg tgc act agt cgt acg ttt gat ctc aag ctt ggt ccc SEQ ID NO: 227).

The following PCR program is used for amplification: denaturation at 94°C for 20 sec; primer annealing at 52°C for 50 sec and primer extension at 72°C for 60 sec and 40 cycles, followed by a 10 min final extension at 72°C.

450 ng of the kappa light chain fragments (SacI-SpeI digested) are ligated with 1400 ng of the phagemid pComb3H5BHis (SacI-SpeI digested; large fragment). The resulting combinatorial antibody library is then transformed into 300 µl of electrocompetent *Escherichia coli* XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm, Biorad gene-pulser) resulting in a library size of more than 10^7 independent clones. After one hour of phenotype expression, positive transformants are selected for carbenicillin resistance encoded by the pComb3H5BHis vector in 100 ml of liquid super broth (SB)-culture over night. Cells are then harvested by centrifugation and plasmid preparation is carried out using a commercially available plasmid preparation kit (Qiagen).

2800 ng of this plasmid-DNA containing the VK-library (XhoI-BstEII digested; large fragment) are ligated with 900 ng of the heavy chain V-fragments (XhoI-BstEII digested) and again transformed into two 300 µl aliquots of electrocompetent *E. coli* XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm) resulting in a total VH-VK scFv (single chain variable fragment) library size of more than 10^7 independent clones.

After phenotype expression and slow adaptation to carbenicillin, the *E. coli* cells containing the antibody library are transferred into SB-Carbenicillin (SB with 50 µg/mL carbenicillin) selection medium. The *E. coli* cells containing the antibody library are then infected with an infectious dose of 10^{12} particles of helper phage VCSM13 resulting in the production and secretion of filamentous M13 phage, wherein each phage particle contains single stranded pComb3H5BHis-DNA encoding a murine scFv-fragment and displays the corresponding scFv-protein as a translational fusion to phage coat protein III. This pool of phages displaying the antibody library is later used for the selection of antigen binding entities.

2.8 Phage display based selection of membrane-proximal target binders on CHO cells expressing the mutated human PSMA antigen with rat membrane-distal epitopes

The phage library carrying the cloned scFv-repertoire is harvested from the
5 respective culture supernatant by PEG8000/NaCl precipitation and centrifugation.
Approximately 10^{11} to 10^{12} scFv phage particles are resuspended in 0.4 ml of
PBS/0.1% BSA and incubated with 10^5 to 10^7 CHO cells expressing the mutated
human PSMA antigen with rat membrane-distal epitopes as described in example 2.4
for 1 hour on ice under slow agitation. These CHO cells are grown beforehand,
10 harvested by centrifugation, washed in PBS and resuspended in PBS/1 % FCS
(containing Na Azide). scFv phage which do not specifically bind to the CHO cells are
eliminated by up to five washing steps with PBS/1 % FCS (containing Na Azide).
After washing, binding entities are eluted from the cells by resuspending the cells in
HCl-glycine pH 2.2 (10 min incubation with subsequent vortexing) and after
15 neutralization with 2 M Tris pH 12, the eluate is used for infection of a fresh
uninfected E. coli XL1 Blue culture ($OD_{600} > 0.5$). The E. coli culture containing E.
coli cells successfully transduced with a phagemid copy, encoding a murine scFv-
fragment, are again selected for carbenicillin resistance and subsequently infected
with VCMS 13 helper phage to start the second round of antibody display and in vitro
20 selection. Typically a total of 4 to 5 rounds of selections are carried out.

2.9 Screening for membrane-proximal target binders on CHO cells expressing the human PSMA antigen, the rat PSMA antigen and the mutated rat PSMA antigen with human membrane-distal epitopes

Plasmid DNA corresponding to 4 and 5 rounds of panning is isolated from E. coli
25 cultures after selection. For the production of soluble scFv-protein, VH-VL-DNA
fragments are excised from the plasmids (XhoI-SpeI). These fragments are cloned
via the same restriction sites in the plasmid pComb3H5BFlag/His differing from the
original pComb3H5BHis in that the expression construct (e.g. scFv) includes a Flag-
tag (TG DYK D D D D K) between the scFv and the His6-tag and the additional phage
30 proteins are deleted. After ligation, each pool (different rounds of panning) of plasmid
DNA is transformed into 100 μ l heat shock competent E. coli TG1 or XLI blue and
plated onto carbenicillin LB-agar. Single colonies are picked into 100 μ l of LB carb
(LB with 50 μ g/ml carbenicillin).

After induction with 1 mM IPTG E. coli transformed with pComb3H5BFlag/His containing a VL-and VH-segment produce soluble scFv in sufficient amounts. Due to a suitable signal sequence, the scFv is exported into the periplasma where it folds into a functional conformation.

- 5 Single E. coli bacterial colonies from the transformation plates are picked for periplasmic small scale preparations and grown in SB-medium (e.g. 10 ml) supplemented with 20 mM $MgCl_2$ and carbenicillin 50 μ g/ml (and re-dissolved in PBS (e.g. 1 ml) after harvesting. A temperature shock is applied by four rounds of freezing at $-70^{\circ}C$ and thawing at $37^{\circ}C$ whereby the outer membrane of the bacteria is
10 destroyed and the soluble periplasmic proteins including the scFvs are released into the supernatant. After elimination of intact cells and cell-debris by centrifugation, the supernatant containing the murine anti-human PSMA-scFvs is collected and used for further examination.

- Screening of the isolated scFvs for membrane-proximal target binders is performed
15 by flow cytometry on CHO cells expressing the human PSMA antigen as described in Example 2.1, the rat PSMA antigen as described in Example 2.3 and the mutated rat PSMA antigen with human membrane-distal epitopes as described in Example 2.5.

- For flow cytometry 2.5×10^5 cells of the respective cell lines are incubated with 50 μ l supernatant. The binding of the constructs is detected with an anti-His antibody
20 (Penta-His Antibody, BSA free, Qiagen GmbH, Hilden, FRG) at 2 μ g/ml in 50 μ l PBS with 2% FCS. As a second step reagent a R-Phycoerythrin-conjugated affinity purified F(ab')₂ fragment, goat anti-mouse IgG (Fc-gamma fragment specific), diluted 1:100 in 50 μ l PBS with 2% FCS (Dianova, Hamburg, FRG) is used. The samples are measured on a FACScan (BD biosciences, Heidelberg, FRG).

- 25 Only constructs which show binding to CHO cells expressing the human PSMA antigen and do not show binding to CHO cells expressing the rat PSMA antigen and also do not show binding to CHO cells expressing the mutated rat PSMA antigen with human membrane-distal epitopes are selected for further use.

30 **2.10 Generation of human/humanized equivalents of non-human scFvs to membrane-proximal target epitopes of human PSMA**

The VH region of a murine anti-PSMA scFv to a membrane-proximal target epitope of human PSMA is aligned against human antibody germline amino acid sequences. The human antibody germline VH sequence is chosen which has the closest homology to the non-human VH and a direct alignment of the two amino acid

sequences is performed. There are a number of framework residues of the non-human VH that differ from the human VH framework regions ("different framework positions"). Some of these residues may contribute to the binding and activity of the antibody to its target.

- 5 To construct a library that contains the murine CDRs and at every framework position that differs from the chosen human VH sequence both possible residues (the human and the maternal murine amino acid residue), degenerated oligonucleotides are synthesized. These oligonucleotides incorporate at the differing positions the human residue with a probability of 75 % and the murine residue with a probability of 25 %.
- 10 For one human VH e.g. six of these oligonucleotides have to be synthesized that overlap in a terminal stretch of approximately 20 nucleotides. To this end every second primer is an antisense primer. Restriction sites within the oligonucleotides needed for later cloning are deleted.

These primers may have a length of 60 to 90 nucleotides, depending on the number of primers that are needed to span over the whole V sequence.

15 These e.g. six primers are mixed in equal amounts (e.g. 1 µl of each primer (primer stocks 20 to 100 µM) to a 20 µl PCR reaction) and added to a PCR mix consisting of PCR buffer, nucleotides and Taq polymerase. This mix is incubated at 94 °C for 3 minutes, 65 °C for 1 minute, 62°C for 1 minute, 59 °C for 1 minute, 56 °C for 1 minute, 52 °C for 1 minute, 50 °C for 1 minute and at 72°C for 10 minutes in a PCR

20 cycler. Subsequently the product is run in an agarose gel electrophoresis and the product of a size from 200 to 400 base pairs isolated from the gel according to standard methods.

This PCR product is then used as a template for a standard PCR reaction using primers that incorporate suitable N-terminal and C-terminal cloning restriction sites.

25 The DNA fragment of the correct size (for a VH approximately 350 nucleotides) is isolated by agarose gel electrophoresis according to standard methods. In this way sufficient VH DNA fragment is amplified. This VH fragment is now a pool of VH fragments that have each one a different amount of human and murine residues at the respective differing framework positions (pool of humanized VH).

30 The same procedure is performed for the VL region of the murine anti-PSMA scFv to a membrane-proximal target epitope of human PSMA (pool of humanized VL).

The pool of humanized VH is then combined with the pool of humanized VL in the phage display vector pComb3H5Bhis to form a library of functional scFvs from which

– after display on filamentous phage - anti-PSMA binders to membrane-proximal target epitopes of human PSMA are selected, screened, identified and confirmed as described above for the parental non-human (murine) anti-PSMA scFv. Single clones are then analyzed for favorable properties and amino acid sequence. Those scFvs, which are closest in amino acid sequence homology to human germline V-segments, are preferred.

Human/humanized anti-PSMA scFvs to membrane-proximal target epitopes of human PSMA are converted into recombinant bispecific single chain antibodies and further characterized as follows.

2.11 Generation of I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human PSMA

Anti-PSMA scFvs to membrane-proximal target epitopes of human PSMA with favorable properties and amino acid sequence are converted into recombinant bispecific single chain antibodies by joining them via a Gly₄Ser₁-linker with the CD3 specific scFv I2C (SEQ ID NO: 185) to result in constructs with the domain arrangement VH_{PSMA} - (Gly₄Ser₁)₃ - VL_{PSMA} - Ser₁Gly₄Ser₁ - VH_{CD3} - (Gly₄Ser₁)₃ - VL_{CD3}. Alternatively further constructs with different domain arrangements can be generated according to standard protocols. For expression in CHO cells the coding sequences of (i) an N-terminal immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence and (ii) a C-terminal His₆-tag followed by a stop codon are both attached in frame to the nucleotide sequence encoding the bispecific single chain antibodies prior to insertion of the resulting DNA-fragment as obtained by gene synthesis into the multiple cloning site of the expression vector pEF-DHFR (Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

2.12 Expression and purification of bispecific single chain antibody molecules directed at membrane-proximal target epitopes of human PSMA

Bispecific single chain antibody molecules are expressed in Chinese hamster ovary cells (CHO). Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the constructs is induced by addition of increasing concentrations of MTX up to final concentrations of 20 nM MTX. After two passages of stationary culture cell culture supernatant is collected and used in the subsequent experiments. To generate supernatant for purification after two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F-68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively, constructs are transiently expressed in HEK 293 cells. Transfection is performed with 293fectin reagent (Invitrogen, #12347-019) according to the manufacturer's protocol. Furthermore the constructs are alternatively expressed in transiently transfected DHFR deficient CHO cells using for example FuGENE® HD Transfection Reagent (Roche Diagnostics GmbH, Cat. No. 04709691001) according to the manufacturer's protocol.

Äkta® Explorer System (GE Health Systems) and Unicorn® Software are used for chromatography. Immobilized metal affinity chromatography ("IMAC") is performed using a Fractogel EMD chelate® (Merck) which is loaded with ZnCl₂ according to the protocol provided by the manufacturer. The column is equilibrated with buffer A (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl) and the cell culture supernatant (500 ml) is applied to the column (10 ml) at a flow rate of 3 ml/min. The column is washed with buffer A to remove unbound sample. Bound protein is eluted using a two step gradient of buffer B (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl, 0.5 M Imidazole) according to the following procedure:

Step 1: 20% buffer B in 6 column volumes

Step 2: 100% buffer B in 6 column volumes

Eluted protein fractions from step 2 are pooled for further purification. All chemicals are of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

Gel filtration chromatography is performed on a HiLoad 16/60 Superdex 200 prep grade column (GE/Amersham) equilibrated with Equi-buffer (25 mM Citrate, 200 mM

Lysine, 5% Glycerol, pH 7.2). Eluted protein samples (flow rate 1 ml/min) are subjected to standard SDS-PAGE and Western Blot for detection. Prior to purification, the column is calibrated for molecular weight determination (molecular weight marker kit, Sigma MW GF-200). Protein concentrations are determined using OD280 nm.

Purified bispecific single chain antibody protein is analyzed in SDS PAGE under reducing conditions performed with pre-cast 4-12% Bis Tris gels (Invitrogen). Sample preparation and application are performed according to the protocol provided by the manufacturer. The molecular weight is determined with MultiMark protein standard (Invitrogen). The gel is stained with colloidal Coomassie (Invitrogen protocol). The purity of the isolated protein is typically >95% as determined by SDS-PAGE.

The bispecific single chain antibody has a molecular weight of about 52 kDa under native conditions as determined by gel filtration in PBS.

Western Blot is performed using an Optitran® BA-S83 membrane and the Invitrogen Blot Module according to the protocol provided by the manufacturer. The antibody used is directed against the His Tag (Penta His, Qiagen) and a Goat-anti-mouse Ig labeled with alkaline phosphatase (AP) (Sigma) is used as second step reagent, and BCIP/NBT (Sigma) as substrate. A band detected at 52 kD corresponds to purified bispecific single chain antibodies.

2.13 Flow cytometric binding analysis of bispecific antibodies directed at membrane-proximal target epitopes of human PSMA

In order to test the functionality of bispecific antibody constructs regarding the capability to bind to CD3 and to membrane-proximal target epitopes of human PSMA, respectively, a FACS analysis is performed. For this purpose CHO cells transfected with human PSMA as described in Example 2.1 and the human CD3 positive T cell leukemia cell line HPB-ALL (DSMZ, Braunschweig, ACC483) are used. For confirmation of binding to membrane-proximal target epitopes of human PSMA - in addition - CHO cells expressing the rat PSMA antigen as described in Example 2.3 and CHO cells expressing the mutated rat PSMA antigen with human membrane-distal epitopes as described in Example 2.5 are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with 50 µl of cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS).

After washing, bound anti His antibodies are detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is
5 used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Only those constructs that show bispecific binding to human CD3 as well as to
10 human PSMA and neither bind to the rat PSMA antigen nor to the mutated rat PSMA antigen with human membrane-distal epitopes are selected for further use.

2.14 Bioactivity of bispecific antibodies directed at membrane-proximal target epitopes of human PSMA

Bioactivity of generated bispecific single chain antibodies is analyzed by chromium
15 ^{51}Cr release in vitro cytotoxicity assays using the CHO cells transfected with human PSMA described in Example 2.1. As effector cells stimulated human CD4/CD56 depleted PBMC are used.

Stimulated human PBMC are obtained as follows:

A Petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) is coated
20 with a commercially available anti-CD3 specific antibody (e.g. OKT3, Orthoclone) in a final concentration of 1 $\mu\text{g}/\text{ml}$ for 1 hour at 37°C. Unbound protein is removed by one washing step with PBS. The fresh PBMC are isolated from peripheral blood (30 – 50 ml human blood) by Ficoll gradient centrifugation according to standard protocols. 3 - 5 x 10⁷ PBMC are added to the precoated petri dish in 120 ml of RPMI 1640 with
25 stabilized glutamine / 10% FCS / IL-2 20 U/ml (Proleukin, Chiron) and stimulated for 2 days. On the third day the cells are collected and washed once with RPMI 1640. IL 2 is added to a final concentration of 20 U/ml and the cells are cultivated again for one day in the same cell culture medium as above.

By depletion of CD4+ T cells and CD56+ NK cells according to standard protocols
30 CD8+ cytotoxic T lymphocytes (CTLs) are enriched.

Target cells are washed twice with PBS and labeled with 11.1 MBq ^{51}Cr in a final volume of 100 μl RPMI with 50% FCS for 60 minutes at 37°C. Subsequently the labeled target cells are washed 3 times with 5 ml RPMI and then used in the cytotoxicity assay. The assay is performed in a 96 well plate in a total volume of

250µl supplemented RPMI (as above) with an E:T ratio of 10:1. 1 µg/ml of purified bispecific single chain antibody molecule and 20 threefold dilutions thereof are applied. The assay time is 18 hours. Cytotoxicity is measured as relative values of released chromium in the supernatant related to the difference of maximum lysis (addition of Triton-X) and spontaneous lysis (without effector cells). All measurements are done in quadruplicates. Measurement of chromium activity in the supernatants is performed with a Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany). Analysis of the experimental data is performed with Prism 4 for Windows (version 4.02, GraphPad Software Inc., San Diego, California, USA). Sigmoidal dose response curves typically have R^2 values >0.90 as determined by the software. EC50 values calculated by the analysis program are used for comparison of bioactivity.

Only those constructs showing potent recruitment of cytotoxic activity of effector T cells against target cells positive for PSMA are selected for further use.

2.15 Generation of CHO cells expressing macaque PSMA

The cDNA sequence of macaque PSMA is obtained by a set of five PCRs on cDNA from macaque monkey prostate prepared according to standard protocols. The following reaction conditions: 1 cycle at 94°C for 2 minutes followed by 40 cycles with 94°C for 1 minute, 52°C for 1 minute and 72°C for 1.5 minutes followed by a terminal cycle of 72°C for 3 minutes and the following primers are used:

1. forward primer: 5'-cactgtggcccaggttcgagg-3' SEQ ID NO: 228
reverse primer: 5'-gacataccacacaaattcaatacgg-3' SEQ ID NO: 229
2. forward primer: 5'-gctctgctcgcgccgagatgtgg-3' SEQ ID NO: 230
reverse primer: 5'-acgctggacaccacctccagg-3' SEQ ID NO: 231
3. forward primer: 5'-gggtctactgagtgccagagg-3' SEQ ID NO: 232
reverse primer: 5'-actgtgtggctgcttgagc-3' SEQ ID NO: 233
4. forward primer: 5'-gggtgaagtcctatccagatgg-3' SEQ ID NO: 234
reverse primer: 5'-gtgctctgcctgaagcaattcc-3' SEQ ID NO: 235
5. forward primer: 5'-ctcggcttctcttcgggtgg-3' SEQ ID NO: 236
reverse primer: 5'-gcatattcattgtggtgaacctgg-3' SEQ ID NO: 237

Those PCRs generate five overlapping fragments, which are isolated and sequenced according to standard protocols using the PCR primers, and thereby provided a portion of the cDNA sequence coding macaque PSMA from codon 3 to the last codon of the mature protein. To generate a construct for expression of macaque

PSMA a cDNA fragment is obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID NO: 238 and 239). In this construct the coding sequence of macaque PSMA from amino acid 3 to the last amino acid of the mature PSMA protein followed by a stop codon is fused in frame to the coding sequence of the first two amino acids of the human PSMA protein. The gene synthesis fragment is also designed as to contain a Kozak site for eukaryotic expression of the construct and restriction sites at the beginning and the end of the fragment containing the cDNA. The introduced restriction sites, XbaI at the 5' end and Sall at the 3' end, are utilised in the following cloning procedures. The gene synthesis fragment is cloned via XbaI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

2.16 Flow cytometric analysis of cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human PSMA

In order to test the cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human PSMA the capability of the constructs to bind to macaque PSMA and macaque CD3, respectively, is investigated by FACS analysis. For this purpose the macaque PSMA transfected CHO cells as described in example 2.15 and the macaque T cell line 4119LnPx (kindly provided by Prof Fickenscher, Hygiene Institute, Virology, Erlangen-Nuernberg; published in Knappe A, et al., and Fickenscher H., Blood 2000, 95, 3256-61) are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with with 50 µl of cell culture supernatant of transfected cells expressing the cross-species specific bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti His antibodies are detected with an

Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg).

- 5 FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Example 3

3.1. Generation of PSMA- and CD3-directed bispecific single antibodies

- 10 Bispecific single chain antibodies comprising either scFv binding domain P1, P2, P3, P4 or P5 against a PSMA-epitope of $< 60^\circ$ membrane-distance or scFv binding domain D1 or D2 against a PSMA-epitope of $\geq 60^\circ$ membrane-distance and the scFv binding domain I2C directed at CD3epsilon on human T cells were obtained by gene synthesis. The gene synthesis fragments were designed as to contain first a
- 15 Kozak site for eukaryotic expression of the construct, followed by a 19 amino acid immunoglobulin leader peptide, followed in frame by the coding sequence of the bispecific single chain antibody, followed in frame by the coding sequence of a 6 histidine tag and a stop codon. The variable region arrangements as well as the SEQ ID Nos of the cDNA- and amino acid sequences are listed in the table 3 below.

20

Table 3:

SEQ ID (nucl/prot)	Formats of protein constructs (N → C)
281/280	PSMA-P1 LH x I2C HL
295/294	PSMA-P2 LH x I2C HL
309/308	PSMA-P3 LH x I2C HL
323/322	PSMA-P4 LH x I2C HL
337/336	PSMA-P5 LH x I2C HL
351/350	PSMA-D1 LH x I2C HL
365/364	PSMA-D2 LH x I2C HL

The gene synthesis fragments were also designed as to introduce suitable restriction sites at the beginning (EcoRI) and at the end of the fragment (Sal I) for cloning of the

25 gene synthesis fragment into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-

150). The aforementioned procedures were carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence was transfected into DHFR deficient
5 CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct was induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. After two passages of stationary culture cell culture supernatant was
10 collected and used in the subsequent experiments.

3.2. Membrane-distance $< 60\text{\AA}$ or $\geq 60\text{\AA}$ of PSMA-epitopes recognized by I2C-based PSMA-directed bscAbs

Epitope confirmation of PSMA-directed bispecific single antibodies was carried out by flowcytometry on CHO cells transfected with (unmutated) human PSMA, unmutated
15 rat PSMA and rat PSMA mutated to the homologous human amino acid at every mismatched amino acid position with a membrane-distance of $\geq 60\text{\AA}$ as described in Example 2.

200,000 cells of each CHO-transfectant were incubated for 30 min on ice with 50 μl of cell culture supernatant of transfected cells expressing the PSMA-directed
20 bispecific antibody constructs. The cells were washed twice in PBS with 2% FCS and binding of the construct was detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 μl PBS with 2% FCS). After washing, bound anti His antibodies were detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Cell culture medium was used as a
25 negative control. Flowcytometry was performed on a FACS-Calibur apparatus, the CellQuest software was used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity were performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

30 Figure 4 shows, that PSMA-directed bscAbs P1xI2C, P2xI2C, P3xI2C, P4xI2C and P5xI2C bind to (unmutated) human PSMA but neither to unmutated nor to mutated rat PSMA and thus confirms a membrane-distance of $< 60\text{\AA}$ for the PSMA-epitope of each of these bispecific constructs. By contrast, PSMA-directed bscAbs D1xI2C and D2xI2C do not bind to unmutated rat PSMA but to (unmutated) human and mutated

rat PSMA consistent with PSMA-epitopes of a membrane-distance $\geq 60\text{\AA}$.

3.3. Relative T cell cytotoxicity redirected by I2C-based PSMA-directed bscAbs with PSMA-epitopes of a membrane-distance $< 60\text{\AA}$ and $\geq 60\text{\AA}$

T cell cytotoxicity redirected by I2C-based PSMA-directed bscAbs against CHO cells
5 transfected with human PSMA was measured in a chromium 51 (^{51}Cr) release assay. As source of effector T cells stimulated human CD4/CD56 depleted PBMC were used. Stimulated human PBMC were obtained as follows: A Petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) was coated with a commercially available anti-CD3 specific antibody (e.g. OKT3, Orthoclone) in a final concentration
10 of 1 $\mu\text{g/ml}$ for 1 hour at 37°C . Unbound protein was removed by one washing step with PBS. The fresh PBMC were isolated from peripheral blood (30 – 50 ml human blood) by Ficoll gradient centrifugation according to standard protocols. $3 - 5 \times 10^7$ PBMC were added to the precoated petri dish in 120 ml of RPMI 1640 with stabilized glutamine / 10% FCS / IL-2 20 U/ml (Proleukin, Chiron) and stimulated for 2 days. On
15 the third day the cells were collected and washed once with RPMI 1640. IL-2 was added to a final concentration of 20 U/ml and the cells were cultured again for one day in the same cell culture medium as above.

By depletion of CD4+ T cells and CD56+ NK cells according to standard protocols CD8+ cytotoxic T lymphocytes (CTLs) were enriched. Target cells were washed
20 twice with PBS and labelled with 11.1 MBq ^{51}Cr in a final volume of 100 μl RPMI with 50% FCS for 60 minutes at 37°C . Subsequently the labelled target cells were washed 3 times with 5 ml RPMI and then used in the cytotoxicity assay. The assay was performed in a 96 well plate in a total volume of 250 μl supplemented RPMI (as above) with an E:T cell ratio of 10:1. I2C-based PSMA-directed bscAbs were added
25 as culture supernatants from transfected CHO cells at different dilutions. The assay time was 18 hours. Cytotoxicity was measured as relative values of released chromium related to the difference of maximum lysis (addition of Triton-X) and spontaneous lysis (without effector cells). All measurements were carried out in quadruplicates. Measurement of released chromium activity was performed with a
30 Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany). Analysis of the experimental data was performed with Prism 4 for Windows (version 4.02, GraphPad Software Inc., San Diego, California, USA). Sigmoidal dose response curves typically had R^2 values >0.90 as determined by the software.

As shown in Figure 5 all 5 PSMA-directed bscAbs, whose PSMA-epitopes have a membrane-distance of $< 60\text{\AA}$ are substantially more potent in redirecting T cell cytotoxicity than the other two PSMA-directed bscAbs, whose PSMA-epitopes have a membrane-distance of $\geq 60\text{\AA}$.

5 4. Generation of additional bispecific single antibodies directed at CD3 and membrane-proximal target epitopes of human PSMA

The human antibody germline VH sequence VH1 1-03 (<http://vbase.mrc-cpe.cam.ac.uk/>) is chosen as framework context for CDRH1 (SEQ ID NO 260), CDRH2 (SEQ ID NO 261) and CDRH3 (SEQ ID NO 262). For VH1 1-03 the following
 10 degenerated oligonucleotides have to be synthesized that overlap in a terminal stretch of approximately 15-20 nucleotides (to this end every second primer is an antisense primer):

5'P6-VH-A-XhoI (SEQ ID NO: 449)

CTT GAT CTC GAG TCC GGC SCT GAG STG RWG AAG CCT GGC GCC TCC
 15 GTG AAG RTG TCC TGC AAG GCC TCC GGC TAC

3'P6-VH-B (SEQ ID NO: 450)

CCA TTC CAG CMS CTG GCC GGG TKY CTG TYT CAC CCA GTG CAT CAC
 GTA GCC GGT GAA GGT GTA GCC GGA GGC CTT GCA

5'P6-VH-C (SEQ ID NO: 451)

20 CCC GGC CAG SKG CTG GAA TGG ATS GGC TAC ATC AAC CCT TAC AAC
 GAC GTG ACC CGG TAC AAC GGC AAG TTC AAG

3'P6-VH-D (SEQ ID NO: 452)

TTC CAT GTA GGC GGT GGA GGM GKA CKT GTC KCT GGT AAK GGT GRC
 TYT GCC CTT GAA CTT GCC GTT GTA

25 5'P6-VH-E (SEQ ID NO: 453)

TCC ACC GCC TAC ATG GAA CTG TCC RGC CTG ASG TCT GAG GAC ACC
 GCC GTG TAC TAC TGC GCC AGG GGC

3'P6-VH-F-BstEII (SEQ ID NO: 454)

30 CGA TAC GGT GAC CAG AGT GCC TCT GCC CCA GGA GTC GAA GTA GTA
 CCA GTT CTC GCC CCT GGC GCA GTA GTA

This primer-set spans over the whole VH sequence.

Within this set primers are mixed in equal amounts (e.g. 1 μl of each primer (primer stocks 20 to 100 μM) to a 20 μl PCR reaction) and added to a PCR mix consisting of PCR buffer, nucleotides and Taq polymerase. This mix is incubated at 94 $^{\circ}\text{C}$ for 3
 35 minutes, 65 $^{\circ}\text{C}$ for 1 minute, 62 $^{\circ}\text{C}$ for 1 minute, 59 $^{\circ}\text{C}$ for 1 minute, 56 $^{\circ}\text{C}$ for 1 minute, 52 $^{\circ}\text{C}$ for 1 minute, 50 $^{\circ}\text{C}$ for 1 minute and at 72 $^{\circ}\text{C}$ for 10 minutes in a PCR cyclor. Subsequently the product is run in an agarose gel electrophoresis and the product of a size from 200 to 400 isolated from the gel according to standard methods.

The VH PCR product is then used as a template for a standard PCR reaction using primers that incorporate N-terminal and C-terminal suitable cloning restriction sites. The DNA fragment of the correct size (for a VH approximately 350 nucleotides) is isolated by agarose gel electrophoresis according to standard methods. In this way
5 sufficient VH DNA fragment is amplified.

The human antibody germline VL sequence VkII A1 (<http://vbase.mrc-cpe.cam.ac.uk/>) is chosen as framework context for CDRL1 (SEQ ID NO: 255), CDRL2 (SEQ ID NO: 256) and CDRL3 (SEQ ID NO: 257). For VkII A1 the following degenerated oligonucleotides have to be synthesized that overlap in a terminal
10 stretch of approximately 15-20 nucleotides (to this end every second primer is an antisense primer):

5'P6-VL-A-SacI (SEQ ID NO: 455)

CTT GAT GAG CTC GTG ATG ACC CAG TCT CCA SYC TCC CTG SCT GTG
ACT CTG GGC CAG CSG GCC TCC ATC TCT TGC CGG

15 3'P6-VL-B (SEQ ID NO: 456)

CCA GTG CAT GAA GGT GTT GTC GTA GGA GTC GAT GGA CTC GGA GGC
CCG GCA AGA GAT GGA GGC

5'P6-VL-C (SEQ ID NO: 457)

ACC TTC ATG CAC TGG TWT CAG CAG ARG CCT GGC CAG YCT CCT MRC
20 CKG CTG ATC TWC CGG GCC TCT ATC CTG GAA

3'P6-VL-D (SEQ ID NO: 458)

CAG GGT GAA GTC GGT GCC GGA GCC AGA GCC GGA GAA CCG GKC AGG
GAY GCC GGA TTC CAG GAT AGA GGC CCG

5'P6-VL-E (SEQ ID NO: 459)

ACC GAC TTC ACC CTG AMA ATC TMC CST GTG GAG GCC GAS GAC GTG
25 GSC RYC TAC TAC TGC CAC CAG

3'P6-VL-F-BsiWI/SpeI (SEQ ID NO: 460)

ACT CAG ACT AGT CGT ACG CTT GAT TTC CAG CTT GGT CCC TCC GCC
GAA GGT GTA AGG GTC CTC GAT GGA CTG GTG GCA GTA GTA

30 This primer-set spans over the whole corresponding VL sequence.

Within this set primers are mixed in equal amounts (e.g. 1 µl of each primer (primer stocks 20 to 100 µM) to a 20 µl PCR reaction) and added to a PCR mix consisting of PCR buffer, nucleotides and Taq polymerase. This mix is incubated at 94 °C for 3 minutes, 65 °C for 1 minute, 62°C for 1 minute, 59 °C for 1 minute, 56 °C for 1
35 minute, 52 °C for 1 minute, 50 °C for 1 minute and at 72°C for 10 minutes in a PCR cyclor. Subsequently the product is run in an agarose gel electrophoresis and the product of a size from 200 to 400 isolated from the gel according to standard methods.

The VL PCR product is then used as a template for a standard PCR reaction using
40 primers that incorporate N-terminal and C-terminal suitable cloning restriction sites.

The DNA fragment of the correct size (for a VL approximately 330 nucleotides) is isolated by agarose gel electrophoresis according to standard methods. In this way sufficient VL DNA fragment is amplified.

The final VH1 1-03 -based VH PCR product (i.e. the repertoire of human/humanized VH) is combined with the final VkII A1-based VL PCR product (i.e. the repertoire of human/humanized VL) in the phage display vector pComb3H5Bhis. This VH-VL combination forms a library of functional scFvs from which – after display on filamentous phage - anti-PSMA binders are selected, screened, identified and confirmed as described in the following:

- 10 450 ng of the light chain fragments (SacI-SpeI digested) are ligated with 1400 ng of the phagemid pComb3H5Bhis (SacI-SpeI digested; large fragment). The resulting combinatorial antibody library is then transformed into 300 ul of electrocompetent *Escherichia coli* XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 uFD, 200 Ohm, Biorad gene-pulser) resulting in a library size of more than 10^7
- 15 independent clones. After one hour of phenotype expression, positive transformants are selected for carbenicilline resistance encoded by the pComb3H5Bhis vector in 100 ml of liquid super broth (SB)-culture over night. Cells are then harvested by centrifugation and plasmid preparation is carried out using a commercially available plasmid preparation kit (Qiagen).
- 20 2800 ng of this plasmid-DNA containing the VL-library (XhoI-BstEII digested; large fragment) are ligated with 900 ng of the heavy chain V-fragments (XhoI-BstEII digested) and again transformed into two 300 ul aliquots of electrocompetent *E. coli* XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 uFD, 200 Ohm) resulting in a total VH-VL scFv (single chain variable fragment) library size of more
- 25 than 10^7 independent clones.

- After phenotype expression and slow adaptation to carbenicilline, the *E. coli* cells containing the antibody library are transferred into SB-carbenicilline (SB with 50 ug/mL carbenicilline) selection medium. The *E. coli* cells containing the antibody library is then infected with an infectious dose of 10^{12} particles of helper phage
- 30 VCSM13 resulting in the production and secretion of filamentous M13 phage, wherein phage particle contains single stranded pComb3H5Bhis-DNA encoding a scFv-fragment and displayed the corresponding scFv-protein as a translational fusion to phage coat protein III. This pool of phages displaying the antibody library is used for the selection of antigen binding entities.

For this purpose the phage library carrying the cloned scFv-repertoire is harvested from the respective culture supernatant by PEG8000/NaCl precipitation and centrifugation. Approximately 10^{11} to 10^{12} scFv phage particles are resuspended in 0.4 ml of PBS/0.1% BSA and incubated with 10^5 to 10^7 PSMA-positive human prostate cancer cell line LNCaP (ATCC No. CRL-1740) for 1 hour on ice under slow agitation. These LNCaP cells are harvested beforehand by centrifugation, washed in PBS and resuspended in PBS/1 % FCS (containing 0.05% Na Azide). scFv phage which do not specifically bind to LNCaP cells are eliminated by up to five washing steps with PBS/1 % FCS (containing 0.05% Na Azide). After washing, binding entities are eluted from the cells by resuspending the cells in HCl-glycine pH 2.2 (10 min incubation with subsequent vortexing) and after neutralization with 2 M Tris pH 12, the eluate is used for infection of a fresh uninfected E. coli XL1 Blue culture (OD600 > 0.5). The E. coli culture containing E. coli cells successfully transduced with a phagemid copy, encoding a human/humanized scFv-fragment, are again selected for carbenicilline resistance and subsequently infected with VCMS 13 helper phage to start the second round of antibody display and in vitro selection. A total of 4 to 5 rounds of selections are carried out, normally.

In order to screen for PSMA specific binders plasmid DNA corresponding to 4 and 5 rounds of panning is isolated from E. coli cultures after selection. For the production of soluble scFv-protein, VH-VL-DNA fragments are excised from the plasmids (XhoI-SpeI). These fragments are cloned via the same restriction sites into the plasmid pComb3H5BFlag/His differing from the original pComb3H5BHis in that the expression construct (i.e. the scFv) includes a Flag-tag (DYKDDDDK) at its C-terminus before the His6-tag and that phage protein III/N2 domain and protein III/CT domain had been deleted. After ligation, each pool (different rounds of panning) of plasmid DNA is transformed into 100 µl heat shock competent E. coli TG1 or XLI blue and plated onto carbenicilline LB-agar. Single colonies are picked into 100 µl of LB carb (50 µg/ml carbenicilline).

E. coli transformed with pComb3H5BFlag/His containing a VL-and VH-segment produce soluble scFv in sufficient amounts after induction with 1 mM IPTG. Due to a suitable signal sequence, the scFv-chain is exported into the periplasma where it folds into a functional conformation.

Single E. coli TG1 bacterial colonies from the transformation plates are picked for periplasmic small scale preparations and grown in SB-medium (e.g. 10 ml)

supplemented with 20 mM $MgCl_2$ and carbenicilline 50 μ g/ml (and re-dissolved in PBS (e.g. 1 ml) after harvesting. By four rounds of freezing at $-70^\circ C$ and thawing at $37^\circ C$, the outer membrane of the bacteria is destroyed by temperature shock and the soluble periplasmic proteins including the scFvs are released into the supernatant.

- 5 After elimination of intact cells and cell-debris by centrifugation, the supernatant containing the anti-PSMA scFvs is collected and used for the identification of PSMA specific binders as follows:

Binding of scFvs to PSMA is tested by flow cytometry on the PSMA-positive human prostate cancer cell line LNCaP (ATCC No. CRL-1740). A periplasmic small scale preparation as described above without any grown bacteria is used as negative control.

- 10 For flow cytometry 2.5×10^5 cells are incubated with 50 μ l of scFv periplasmic preparation or with 5 μ g/ml of purified scFv in 50 μ l PBS with 2% FCS. The binding of scFv is detected with an anti-His antibody (Penta-His Antibody, BSA free, Qiagen GmbH, Hilden, FRG) at 2 μ g/ml in 50 μ l PBS with 2% FCS. As a second step reagent
- 15 a R-Phycoerythrin-conjugated affinity purified F(ab')₂ fragment, goat anti-mouse IgG (Fc-gamma fragment specific), diluted 1:100 in 50 μ l PBS with 2% FCS (Dianova, Hamburg, FRG) is used. The samples are measured on a FACScan (BD biosciences, Heidelberg, FRG).

- 20 Single clones are then analyzed for favourable properties and amino acid sequence. PSMA specific scFvs are converted into recombinant bispecific single chain antibodies by joining them via a Gly₄Ser₁-linker with the CD3 specific scFv I2C (SEQ ID NO: 185) or any other CD3 specific scFv of the invention to result in constructs with the domain arrangement VH_{PSMA} - (Gly₄Ser₁)₃ - VL_{PSMA} - Ser₁Gly₄Ser₁-VH_{CD3} -
- 25 (Gly₄Ser₁)₃ - VL_{CD3} or alternative domain arrangements such as VL_{PSMA} - (Gly₄Ser₁)₃ - VH_{PSMA} - Gly₄Ser₁-VH_{CD3} - (Gly₄Ser₁)₃ - VL_{CD3}. For expression in CHO cells the coding sequences of (i) an N-terminal immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence and (ii) a C-terminal His₆-tag followed by a stop codon are both attached in frame to the nucleotide
- 30 sequence encoding the bispecific single chain antibodies prior to insertion of the resulting DNA-fragment as obtained by gene synthesis into the multiple cloning site of the expression vector pEF-DHFR (Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). Transfection of the generated expression plasmids is carried out as described in Example 3.1. Protein expression and purification of bispecific antibody

constructs, flow cytometric confirmation of binding to CD3 and to membrane-proximal target epitopes of human PSMA as well as the analysis of bioactivity by cytotoxicity assay are performed as described in Example 2. All other state of the art procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)).

Only those bispecific antibody constructs that bind to CD3 and to membrane-proximal target epitopes of human PSMA and show potent recruitment of cytotoxic activity of effector T cells against target cells positive for PSMA are selected for further use.

Example 4

4.1. Generation of PSMA- and CD3-directed bispecific single chain antibodies

Bispecific single chain antibodies comprising either scFv binding domain P6 against a PSMA-epitope of < 60Å membrane-distance or scFv binding domain D3 against a PSMA-epitope of ≥ 60Å membrane-distance and the scFv binding domain I2C directed at CD3epsilon on human T cells were obtained by gene synthesis. The gene synthesis fragments were designed as to contain first a Kozak site for eukaryotic expression of the construct, followed by a 19 amino acid immunoglobulin leader peptide, followed in frame by the coding sequence of the bispecific single chain antibody, followed in frame by the coding sequence of a 6 histidine tag and a stop codon. The variable region arrangements as well as the SEQ ID Nos of the cDNA- and amino acid sequences are listed in the table 4 below.

Table 4:

SEQ ID (nucl/prot)	Formats of protein constructs (N → C)
267/266	PSMA-P6 LH x I2C HL
253/252	PSMA-D3 LH x I2C HL

The gene synthesis fragments were also designed as to introduce suitable restriction sites at the beginning (EcoRI) and at the end of the fragment (Sal I) for cloning of the gene synthesis fragment into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures were carried out according to standard

protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence was transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct was induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. After two passages of stationary culture cell culture supernatant was collected and used in the subsequent experiments.

4.2. Membrane-distance $< 60\text{\AA}$ or $\geq 60\text{\AA}$ of PSMA-epitopes recognized by I2C-based PSMA-directed bscAbs

Epitope confirmation of PSMA-directed bispecific single antibodies was carried out by flowcytometry on CHO cells transfected with (unmutated) human PSMA, unmutated rat PSMA and rat PSMA mutated to the homologous human amino acid at every mismatched amino acid position with a membrane-distance of $\geq 60\text{\AA}$ as described in Example 2.

200,000 cells of each CHO-transfectant were incubated for 30 min on ice with 50 μl of cell culture supernatant of transfected cells expressing the PSMA-directed bispecific antibody constructs. The cells were washed twice in PBS with 2% FCS and binding of the construct was detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 μl PBS with 2% FCS). After washing, bound anti His antibodies were detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Cell culture medium was used as a negative control. Flowcytometry was performed on a FACS-Calibur apparatus, the CellQuest software was used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity were performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Figure 6 shows, that PSMA-directed bscAb P6xI2C binds to (unmutated) human PSMA but neither to unmutated nor to mutated rat PSMA and thus confirms a membrane-distance of $< 60\text{\AA}$ for the PSMA-epitope of this bispecific construct. By contrast, PSMA-directed bscAb D3xI2C does not bind to unmutated rat PSMA but to (unmutated) human and mutated rat PSMA consistent with a PSMA-epitope of a membrane-distance $\geq 60\text{\AA}$.

4.3. Relative T cell cytotoxicity redirected by I2C-based PSMA-directed bscAbs with PSMA-epitopes of a membrane-distance < 60Å and ≥ 60Å

T cell cytotoxicity redirected by I2C-based PSMA-directed bscAbs against CHO cells transfected with human PSMA was measured in a chromium 51 (⁵¹Cr) release assay.

5 As source of effector T cells stimulated human CD4/CD56 depleted PBMC were used. Stimulated human PBMC were obtained as follows: A Petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) was coated with a commercially available anti-CD3 specific antibody (e.g. OKT3, Orthoclone) in a final concentration of 1 µg/ml for 1 hour at 37°C. Unbound protein was removed by one washing step
10 with PBS. The fresh PBMC were isolated from peripheral blood (30 – 50 ml human blood) by Ficoll gradient centrifugation according to standard protocols. 3 - 5 x 10⁷ PBMC were added to the precoated petri dish in 120 ml of RPMI 1640 with stabilized glutamine / 10% FCS / IL-2 20 U/ml (Proleukin, Chiron) and stimulated for 2 days. On the third day the cells were collected and washed once with RPMI 1640. IL-2 was
15 added to a final concentration of 20 U/ml and the cells were cultured again for one day in the same cell culture medium as above.

By depletion of CD4+ T cells and CD56+ NK cells according to standard protocols CD8+ cytotoxic T lymphocytes (CTLs) were enriched. Target cells were washed twice with PBS and labelled with 11.1 MBq ⁵¹Cr in a final volume of 100µl RPMI with
20 50% FCS for 60 minutes at 37°C. Subsequently the labelled target cells were washed 3 times with 5 ml RPMI and then used in the cytotoxicity assay. The assay was performed in a 96 well plate in a total volume of 250µl supplemented RPMI (as above) with an E:T cell ratio of 10:1. I2C-based PSMA-directed bscAbs were added as culture supernatants from transfected CHO cells at different dilutions. The assay
25 time was 18 hours. Cytotoxicity was measured as relative values of released chromium related to the difference of maximum lysis (addition of Triton-X) and spontaneous lysis (without effector cells). All measurements were carried out in quadruplicates. Measurement of released chromium activity was performed with a Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany).
30 Analysis of the experimental data was performed with Prism 4 for Windows (version 4.02, GraphPad Software Inc., San Diego, California, USA). Sigmoidal dose response curves typically had R² values >0.90 as determined by the software.

As shown in Figure 7 the PSMA-directed bscAb P6xI2C, whose PSMA-epitope has a membrane-distance of < 60Å is substantially more potent in redirecting T cell

cytotoxicity than PSMA-directed bscAb D3xI2C, whose PSMA-epitope has a membrane-distance of $\geq 60\text{\AA}$.

Example 5: Crossreactive binding to human and non-chimpanzee primate PSMA and CD3 of I2C-based bscAbs against membrane-proximal PSMA-epitopes

5.1. Cloning and expression of cyno PSMA antigen on CHO cells

The cDNA sequence of macaque PSMA was obtained as described in Example 2.15. As described above, these PCRs generated five overlapping fragments, which were isolated and sequenced according to standard protocols using the PCR primers, and thereby provided a portion of the cDNA sequence coding macaque PSMA from codon 3 to the last codon of the mature protein. To generate a construct for expression of macaque PSMA a cDNA fragment was obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 238 and 239). In this construct the coding sequence of macaque PSMA from amino acid 3 to the last amino acid of the mature PSMA protein followed by a stop codon was fused in frame to the coding sequence of the first two amino acids of the human PSMA protein. The gene synthesis fragment was also designed as to contain a Kozak site for eukaryotic expression of the construct and restriction sites at the beginning and the end of the fragment containing the cDNA. The introduced restriction sites, XbaI at the 5' end and Sall at the 3' end, were utilised in the following cloning procedures. The gene synthesis fragment was cloned via XbaI and Sall into a plasmid designated pEF-DHFR following standard protocols. The aforementioned procedures were carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence was transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct was induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

5.2. Flow cytometric binding analysis of I2C-based bscAbs against membrane-proximal epitopes of human PSMA on non-chimpanzee primate PSMA and on human and non-chimpanzee primate CD3

Binding of bscAbs P1xI2C, P2xI2C, P3xI2C, P4xI2C, P5xI2C and P6xI2C directed
5 against membrane-proximal PSMA-epitopes to CHO cells expressing human PSMA
is shown by flowcytometry in Examples 3 and 4.

Binding of these bscAbs to macaque PSMA as well as to human and macaque CD3
was analysed by flowcytometry using CHO cells transfected with macaque PSMA,
the human CD3 positive T cell leukemia cell line HPB-ALL (DSMZ, Braunschweig,
10 ACC483) and the CD3 positive macaque T cell line 4119LnPx (kindly provided by
Prof Fickenscher, Hygiene Institute, Virology, Erlangen-Nuernberg; published in
Knappe A, et al., and Fickenscher H., Blood 2000, 95, 3256-61). Results are shown
in Figure 8. 200,000 cells of the respective cell population were incubated for 30 min
on ice with 50 µl of cell culture supernatant of CHO cells transfected with the PSMA-
15 directed bispecific antibody constructs. The cells were washed twice in PBS and
binding of the construct was detected with an unlabeled murine Penta His antibody
(Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti His
antibodies were detected with an Fc gamma-specific antibody (Dianova) conjugated
to phycoerythrin, diluted 1:100 in 50 µl PBS with 2% FCS. Fresh culture medium was
20 used as a negative control.

Flow cytometry was performed on a FACS-Calibur apparatus, the CellQuest software
was used to acquire and analyze the data (Becton Dickinson biosciences,
Heidelberg). FACS staining and measuring of the fluorescence intensity were
performed as described in Current Protocols in Immunology (Coligan, Kruisbeek,
25 Margulies, Shevach and Strober, Wiley-Interscience, 2002).

6. Generation of bispecific single chain antibodies directed at membrane-proximal target epitopes of human FAPalpha

6.1 Generation of CHO cells expressing human FAPalpha

The coding sequence of human FAPalpha as published in GenBank (Accession
30 number NM_004460) is obtained by gene synthesis according to standard protocols.
The gene synthesis fragment is designed as to contain first a Kozak site for
eukaryotic expression of the construct followed by the coding sequence of the human
FAPalpha protein and a stop codon (the cDNA and amino acid sequence of the
construct is listed under SEQ ID Nos 366 and 367). The gene synthesis fragment is

also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, XmaI at the 5' end and Sall at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via XmaI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

6.2 Generation of a soluble human FAPalpha fusion protein

The coding sequence of human FAPalpha as described in Example 6.1 and the coding sequence of murine Lag3 as published in GenBank (Accession number NM_008479) are used for the construction of an artificial cDNA sequence encoding a soluble fusion protein of human FAPalpha and murine Lag3. To generate a construct for expression of the soluble human FAPalpha fusion protein a cDNA fragment is obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 368 369). The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the murine Lag3 protein from amino acid 1 to 441 corresponding to the signal peptide and extracellular domains of murine Lag3, followed in frame by the coding sequence of an artificial Ser₁-Gly₄-Ser₁-linker, followed in frame by the coding sequence of the human FAPalpha protein from amino acid 27 to 760 corresponding to the extracellular domains of human FAPalpha, followed in frame by the coding sequence of an artificial Ser₁-Gly₁-linker, followed in frame by the coding sequence of a 6 histidine tag and a stop codon. The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, SpeI at the 5' end and Sall at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via SpeI

and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are all carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. After two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F – 68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively a clone of the expression plasmid with sequence-verified nucleotide sequence is used for transfection and protein expression in the FreeStyle 293 Expression System (Invitrogen GmbH, Karlsruhe, Germany) according to the manufacturer's protocol. Supernatant containing the expressed protein is obtained, cells are removed by centrifugation and the supernatant is stored at -20°C.

Purification of the soluble human FAPalpha fusion protein is performed as follows: Äkta® Explorer System (GE Health Systems) and Unicorn® Software are used for chromatography. Immobilized metal affinity chromatography ("IMAC") is performed using a Fractogel EMD chelate® (Merck) which is loaded with ZnCl₂ according to the protocol provided by the manufacturer. The column is equilibrated with buffer A (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl) and the cell culture supernatant (500 ml) is applied to the column (10 ml) at a flow rate of 3 ml/min. The column is washed with buffer A to remove unbound sample. Bound protein is eluted using a two step gradient of buffer B (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl, 0.5 M Imidazole) according to the following procedure:

Step 1: 20% buffer B in 6 column volumes
Step 2: 100% buffer B in 6 column volumes

Eluted protein fractions from step 2 are pooled for further purification. All chemicals are of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

Gel filtration chromatography is performed on a HiLoad 16/60 Superdex 200 prep grade column (GE/Amersham) equilibrated with Equi-buffer (25 mM Citrate, 200 mM Lysine, 5% Glycerol, pH 7.2). Eluted protein samples (flow rate 1 ml/min) are subjected to standard SDS-PAGE and Western Blot for detection. Prior to
5 purification, the column is calibrated for molecular weight determination (molecular weight marker kit, Sigma MW GF-200). Protein concentrations are determined using OD280 nm.

6.3 Generation of CHO cells expressing murine FAPalpha

The sequence of murine FAPalpha (NM_007986, Mus musculus fibroblast activation protein (Fap), mRNA, National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/entrez>) is used to obtain a synthetic cDNA molecule by
10 gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the complete murine FAPalpha antigen, followed
15 in frame by the coding sequence of a FLAG-tag and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 370 and 371). An alternative construct identical to the aforementioned construct except for a C-terminal 6 Histidine-tag instead of the FLAG-tag is also generated. The gene synthesis
20 fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring
25 Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing
30 concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. Cell surface expression of murine FAPalpha by the generated transfectants is confirmed by flow cytometric binding analysis performed as described herein. In the case of the construct with the 6 Histidine tag a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS) was used and detected with an Fc gamma-

specific antibody (Dianova) conjugated to phycoerythrin. Expression of murine FAPalpha was confirmed as shown in Figure 10.

6.4 Generation of CHO cells expressing a mutated human FAPalpha antigen with murine membrane-distal epitopes

- 5 The coding sequence of a mutated human FAPalpha antigen with murine membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the complete human FAPalpha antigen mutated at 15 specific amino acid positions as explained below,
- 10 followed in frame by the coding sequence of a FLAG-tag and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 372 and 373). All extracellular amino acids of human FAPalpha mismatching with the homologous murine sequence, whose alpha C-atoms have a distance of ≥ 60 Å from the alpha C-atom of the thirteenth extracellular amino acid (i.e. the reference aa) as
- 15 counted from the junction of transmembrane and extracellular region, are mutated to the homologous mismatched murine amino acid. This applies to the 15 amino acids that are listed in table 2 and marked in bold. The homologous mismatched amino acids between human and murine FAPalpha are identified by sequence alignment as shown in Figure 9. The gene synthesis fragment is also designed as to introduce
- 20 restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). Internal restriction sites are removed by silent mutation of the coding sequence in the gene synthesis fragment. The aforementioned procedures are carried out according to
- 25 standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990)
- 30 Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

Cell surface expression of mutated human FAPalpha with murine membrane-distal epitopes by the generated transfectants is confirmed by flow cytometric binding

analysis performed as described herein using an anti-FLAG M2 antibody (Sigma-Aldrich, Inc.; diluted 1:900 in 50 µl PBS with 2% FCS) detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin. Expression of mutated human FAPalpha with murine membrane-distal epitopes was confirmed as shown in
5 Figure 10.

6.5 Generation of CHO cells expressing a mutated murine FAPalpha antigen with human membrane-distal epitopes

The coding sequence of a mutated murine FAPalpha antigen with human membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The
10 gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the complete murine FAPalpha antigen mutated at the same 15 specific extracellular amino acid positions as identified in the foregoing Example 6.4 to the respective homologous human amino acid, followed in frame by the coding sequence of a FLAG tag and a stop
15 codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 374 and 375). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The
20 aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR
25 deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

6.6 Immunization of mice using a soluble human FAPalpha fusion protein

30 Twelve weeks old F1 mice from BALB/c x C57BL/6 crossings are immunized with the soluble human FAPalpha fusion protein as described in Example 6.2. To this end for each animal 40 µg of the soluble human FAPalpha fusion protein are mixed with 10 nmol of a thioate-modified CpG-Oligonucleotide (5'-tccatgacgttctctgatgct-3') in

300 µl PBS and are injected intraperitoneally. Mice receive booster immunizations after 21, 42 and optionally 63 days in the same way. Ten days after the first booster immunization, blood samples are taken and antibody serum titers against human FAPalpha are tested by flow cytometry according to standard protocols. To this end
5 200.000 cells of the human FAPalpha transfected CHO cells as described in Example 6.1 are incubated for 30 min on ice with 50 µl of serum of the immunized animals diluted 1:1000 in PBS with 2% FCS. The cells are washed twice in PBS with 2% FCS and binding of serum antibodies is detected with a mouse Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with
10 2% FCS. Serum of the animals obtained prior to immunization is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as
15 described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Animals demonstrating significant serum reactivity against human FAPalpha as determined by the FACS analysis are used in the subsequent experiment.

6.7 Generation of an immune murine antibody scFv library: Construction of a 20 combinatorial antibody library and phage display

Three days after the last booster immunization spleen cells of reactive animals are harvested for the preparation of total RNA according to standard protocols.

A library of murine immunoglobulin (Ig) light chain (kappa) variable region (VK) and Ig heavy chain variable region (VH) DNA-fragments is constructed by RT-PCR on
25 murine spleen RNA using VK-and VH specific primers. cDNA is synthesized according to standard protocols, see example 2.7.

450 ng of the kappa light chain fragments (SacI-SpeI digested) are ligated with 1400 ng of the phagemid pComb3H5Bhis (SacI-SpeI digested; large fragment). The resulting combinatorial antibody library is then transformed into 300 µl of
30 electrocompetent Escherichia coli XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm, Biorad gene-pulser) resulting in a library size of more than 10^7 independent clones. After one hour of phenotype expression, positive transformants are selected for carbenicillin resistance encoded by the pComb3H5BHis vector in 100 ml of liquid super broth (SB)-culture over night. Cells

are then harvested by centrifugation and plasmid preparation is carried out using a commercially available plasmid preparation kit (Qiagen).

2800 ng of this plasmid-DNA containing the VK-library (XhoI-BstEII digested; large fragment) are ligated with 900 ng of the heavy chain V-fragments (XhoI-BstEII digested) and again transformed into two 300 µl aliquots of electrocompetent E. coli XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm) resulting in a total VH-VK scFv (single chain variable fragment) library size of more than 10^7 independent clones.

After phenotype expression and slow adaptation to carbenicillin, the E. coli cells containing the antibody library are transferred into SB-Carbenicillin (SB with 50 µg/mL carbenicillin) selection medium. The E. coli cells containing the antibody library are then infected with an infectious dose of 10^{12} particles of helper phage VCSM13 resulting in the production and secretion of filamentous M13 phage, wherein each phage particle contains single stranded pComb3H5BHis-DNA encoding a murine scFv-fragment and displays the corresponding scFv-protein as a translational fusion to phage coat protein III. This pool of phages displaying the antibody library is later used for the selection of antigen binding entities.

6.8 Phage display based selection of membrane-proximal target binders on CHO cells expressing the mutated human FAPalpha antigen with murine membrane-distal epitopes

The phage library carrying the cloned scFv-repertoire is harvested from the respective culture supernatant by PEG8000/NaCl precipitation and centrifugation. Approximately 10^{11} to 10^{12} scFv phage particles are resuspended in 0.4 ml of PBS/0.1% BSA and incubated with 10^5 to 10^7 CHO cells expressing the mutated human FAPalpha antigen with murine membrane-distal epitopes as described in example 6.4 for 1 hour on ice under slow agitation. These CHO cells are grown beforehand, harvested by centrifugation, washed in PBS and resuspended in PBS/1 % FCS (containing Na Azide). scFv phage which do not specifically bind to the CHO cells are eliminated by up to five washing steps with PBS/1 % FCS (containing Na Azide). After washing, binding entities are eluted from the cells by resuspending the cells in HCl-glycine pH 2.2 (10 min incubation with subsequent vortexing) and after neutralization with 2 M Tris pH 12, the eluate is used for infection of a fresh uninfected E. coli XL1 Blue culture (OD600 > 0.5). The E. coli culture containing E. coli cells successfully transduced with a phagemid copy, encoding a murine scFv-

fragment, are again selected for carbenicillin resistance and subsequently infected with VCMS 13 helper phage to start the second round of antibody display and in vitro selection. Typically a total of 4 to 5 rounds of selections are carried out.

6.9 Screening for membrane-proximal target binders on CHO cells expressing the human FAPalpha antigen, the murine FAPalpha antigen and the mutated murine FAPalpha antigen with human membrane-distal epitopes

Plasmid DNA corresponding to 4 and 5 rounds of panning is isolated from E. coli cultures after selection. For the production of soluble scFv-protein, VH-VL-DNA fragments are excised from the plasmids (XhoI-SpeI). These fragments are cloned via the same restriction sites in the plasmid pComb3H5BFlag/His differing from the original pComb3H5BHis in that the expression construct (e.g. scFv) includes a Flag-tag (TG DYKDDDDK) between the scFv and the His6-tag and the additional phage proteins are deleted. After ligation, each pool (different rounds of panning) of plasmid DNA is transformed into 100 µl heat shock competent E. coli TG1 or XLI blue and plated onto carbenicillin LB-agar. Single colonies are picked into 100 µl of LB carb (LB with 50 µg/ml carbenicillin).

After induction with 1 mM IPTG E. coli transformed with pComb3H5BFlag/His containing a VL- and VH-segment produce soluble scFv in sufficient amounts. Due to a suitable signal sequence, the scFv is exported into the periplasma where it folds into a functional conformation.

Single E. coli bacterial colonies from the transformation plates are picked for periplasmic small scale preparations and grown in SB-medium (e.g. 10 ml) supplemented with 20 mM MgCl₂ and carbenicillin 50 µg/ml (and re-dissolved in PBS (e.g. 1 ml) after harvesting. A temperature shock is applied by four rounds of freezing at -70°C and thawing at 37°C whereby the outer membrane of the bacteria is destroyed and the soluble periplasmic proteins including the scFvs are released into the supernatant. After elimination of intact cells and cell-debris by centrifugation, the supernatant containing the murine anti-human FAPalpha-scFvs is collected and used for further examination.

Screening of the isolated scFvs for membrane-proximal target binders is performed by flow cytometry on CHO cells expressing the human FAPalpha antigen as described in Example 6.1, the murine FAPalpha antigen as described in Example 6.3 and the mutated murine FAPalpha antigen with human membrane-distal epitopes as described in Example 6.5.

For flow cytometry 2.5×10^5 cells of the respective cell lines are incubated with 50 μ l supernatant. The binding of the constructs is detected with an anti-His antibody (Penta-His Antibody, BSA free, Qiagen GmbH, Hilden, FRG) at 2 μ g/ml in 50 μ l PBS with 2% FCS. As a second step reagent an R-Phycoerythrin-conjugated affinity
5 purified F(ab')₂ fragment, goat anti-mouse IgG (Fc-gamma fragment specific), diluted 1:100 in 50 μ l PBS with 2% FCS (Dianova, Hamburg, FRG) is used. The samples are measured on a FACSScan (BD biosciences, Heidelberg, FRG).

Only constructs which show binding to CHO cells expressing the human FAPalpha antigen and do not show binding to CHO cells expressing the murine FAPalpha
10 antigen and also do not show binding to CHO cells expressing the mutated murine FAPalpha antigen with human membrane-distal epitopes are selected for further use.

6.10 Generation of human/humanized equivalents of non-human scFvs to membrane-proximal target epitopes of human FAPalpha

The VH region of a murine anti-FAPalpha scFv to a membrane-proximal target
15 epitope of human FAPalpha is aligned against human antibody germline amino acid sequences. The human antibody germline VH sequence is chosen which has the closest homology to the non-human VH and a direct alignment of the two amino acid sequences is performed. There are a number of framework residues of the non-human VH that differ from the human VH framework regions ("different framework
20 positions"). Some of these residues may contribute to the binding and activity of the antibody to its target.

To construct a library that contains the murine CDRs and at every framework position that differs from the chosen human VH sequence both possible residues (the human and the maternal murine amino acid residue), degenerated oligonucleotides are
25 synthesized. These oligonucleotides incorporate at the differing positions the human residue with a probability of 75 % and the murine residue with a probability of 25 %. For one human VH e.g. six of these oligonucleotides have to be synthesized that overlap in a terminal stretch of approximately 20 nucleotides. To this end every second primer is an antisense primer. Restriction sites within the oligonucleotides
30 needed for later cloning are deleted.

These primers may have a length of 60 to 90 nucleotides, depending on the number of primers that are needed to span over the whole V sequence.

These e.g. six primers are mixed in equal amounts (e.g. 1 µl of each primer (primer stocks 20 to 100 µM) to a 20 µl PCR reaction) and added to a PCR mix consisting of PCR buffer, nucleotides and Taq polymerase. This mix is incubated at 94 °C for 3 minutes, 65 °C for 1 minute, 62°C for 1 minute, 59 °C for 1 minute, 56 °C for 1 minute, 52 °C for 1 minute, 50 °C for 1 minute and at 72°C for 10 minutes in a PCR
5 cycler. Subsequently the product is run in an agarose gel electrophoresis and the product of a size from 200 to 400 base pairs isolated from the gel according to standard methods.

This PCR product is then used as a template for a standard PCR reaction using
10 primers that incorporate suitable N-terminal and C-terminal cloning restriction sites. The DNA fragment of the correct size (for a VH approximately 350 nucleotides) is isolated by agarose gel electrophoresis according to standard methods. In this way sufficient VH DNA fragment is amplified. This VH fragment is now a pool of VH fragments that have each one a different amount of human and murine residues at
15 the respective differing framework positions (pool of humanized VH). The same procedure is performed for the VL region of the murine anti-FAPalpha scFv to a membrane-proximal target epitope of human FAPalpha (pool of humanized VL).

The pool of humanized VH is then combined with the pool of humanized VL in the phage display vector pComb3H5Bhis to form a library of functional scFvs from which
20 – after display on filamentous phage - anti-FAPalpha binders to membrane-proximal target epitopes of human FAPalpha are selected, screened, identified and confirmed as described above for the parental non-human (murine) anti-FAPalpha scFv. Single clones are then analyzed for favorable properties and amino acid sequence. Those scFvs, which are closest in amino acid sequence homology to human germline V-
25 segments, are preferred.

Human/humanized anti-FAPalpha scFvs to membrane-proximal target epitopes of human FAPalpha are converted into recombinant bispecific single chain antibodies and further characterized as follows.

6.11 Generation of I2C-based bispecific single chain antibodies directed at 30 membrane-proximal target epitopes of human FAPalpha

Anti-FAPalpha scFvs to membrane-proximal target epitopes of human FAPalpha with favorable properties and amino acid sequence are converted into recombinant bispecific single chain antibodies by joining them via a Gly₄Ser₁-linker with the CD3 specific scFv I2C (SEQ ID NO: 185) to result in constructs with the domain

arrangement $VH_{FAP\alpha} - (Gly_4Ser_1)_3 - VL_{FAP\alpha} - Ser_1Gly_4Ser_1 - VH_{CD3} - (Gly_4Ser_1)_3 - VL_{CD3}$.

I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human FAP α were designed as set out in the following Table 5:

5

Table 5: Formats of I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human FAP α

SEQ ID (nucl/prot)	Formats of protein constructs (N → C)
820/819	FA19D12HLxI2CHL
806/805	FA20H3HLxI2CHL
750/749	FA22A9HLxI2CHL
764/763	FA22C11HLxI2CHL
834/833	FA19D9HLxI2CHL
778/777	FA22D8HLxI2CHL
792/791	FA22E8HLxI2CHL

Alternatively further constructs with different domain arrangements can be generated according to standard protocols. For expression in CHO cells the coding sequences of (i) an N-terminal immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence and (ii) a C-terminal His6-tag followed by a stop codon are both attached in frame to the nucleotide sequence encoding the bispecific single chain antibodies prior to insertion of the resulting DNA-fragment as obtained by gene synthesis into the multiple cloning site of the expression vector pEF-DHFR (Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

6.12 Expression and purification of bispecific single chain antibody molecules directed at membrane-proximal target epitopes of human FAP α

Bispecific single chain antibody molecules are expressed in Chinese hamster ovary cells (CHO). Eukaryotic protein expression in DHFR deficient CHO cells is performed

25

as described by Kaufmann R.J. (1990) *Methods Enzymol.* 185, 537-566. Gene amplification of the constructs is induced by addition of increasing concentrations of MTX up to final concentrations of 20 nM MTX. After two passages of stationary culture cell culture supernatant is collected and used in the subsequent experiments.

5 To generate supernatant for purification after two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F-68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively, constructs are transiently
10 expressed in HEK 293 cells. Transfection is performed with 293fectin reagent (Invitrogen, #12347-019) according to the manufacturer's protocol. Furthermore the constructs are alternatively expressed in transiently transfected DHFR deficient CHO cells using for example FuGENE® HD Transfection Reagent (Roche Diagnostics GmbH, Cat. No. 04709691001) according to the manufacturer's protocol.

15 Äkta® Explorer System (GE Health Systems) and Unicorn® Software are used for chromatography. Immobilized metal affinity chromatography ("IMAC") is performed using a Fractogel EMD chelate® (Merck) which is loaded with ZnCl₂ according to the protocol provided by the manufacturer. The column is equilibrated with buffer A (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl) and the cell culture supernatant
20 (500 ml) is applied to the column (10 ml) at a flow rate of 3 ml/min. The column is washed with buffer A to remove unbound sample. Bound protein is eluted using a two step gradient of buffer B (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl, 0.5 M Imidazole) according to the following procedure:

Step 1: 20% buffer B in 6 column volumes

25 Step 2: 100% buffer B in 6 column volumes

Eluted protein fractions from step 2 are pooled for further purification. All chemicals are of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

Gel filtration chromatography is performed on a HiLoad 16/60 Superdex 200 prep
30 grade column (GE/Amersham) equilibrated with Equi-buffer (25 mM Citrate, 200 mM Lysine, 5% Glycerol, pH 7.2). Eluted protein samples (flow rate 1 ml/min) are subjected to standard SDS-PAGE and Western Blot for detection. Prior to purification, the column is calibrated for molecular weight determination (molecular

weight marker kit, Sigma MW GF-200). Protein concentrations are determined using OD280 nm.

Purified bispecific single chain antibody protein is analyzed in SDS PAGE under reducing conditions performed with pre-cast 4-12% Bis Tris gels (Invitrogen). Sample preparation and application are performed according to the protocol provided by the manufacturer. The molecular weight is determined with MultiMark protein standard (Invitrogen). The gel is stained with colloidal Coomassie (Invitrogen protocol). The purity of the isolated protein is typically >95% as determined by SDS-PAGE.

The bispecific single chain antibody has a molecular weight of about 52 kDa under native conditions as determined by gel filtration in PBS.

Western Blot is performed using an Optitran® BA-S83 membrane and the Invitrogen Blot Module according to the protocol provided by the manufacturer. The antibody used is directed against the His Tag (Penta His, Qiagen) and a Goat-anti-mouse Ig labeled with alkaline phosphatase (AP) (Sigma) is used as second step reagent, and BCIP/NBT (Sigma) as substrate. A band detected at 52 kD corresponds to purified bispecific single chain antibodies.

6.13 Flow cytometric binding analysis of bispecific antibodies directed at membrane-proximal target epitopes of human FAPalpha

In order to test the functionality of bispecific antibody constructs regarding the capability to bind to CD3 and to human FAPalpha, respectively, a FACS analysis is performed. For this purpose CHO cells transfected with human FAPalpha as described in Example 6.1 and the human CD3 positive T cell leukemia cell line HPB-ALL (DSMZ, Braunschweig, ACC483) are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with 50 µl of cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti His antibodies are detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as

described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Only those constructs that show bispecific binding to human CD3 as well as to human FAPalpha are selected for further use.

- 5 The bispecific binding of the single chain molecules listed above was clearly detectable as shown in Figure 11. In the FACS analysis all constructs showed binding to human CD3 and human FAPA compared to the negative control.

6.14 Bioactivity of bispecific antibodies directed at membrane-proximal target epitopes of human FAPalpha

- 10 Bioactivity of generated bispecific single chain antibodies is analyzed by chromium 51 (^{51}Cr) release in vitro cytotoxicity assays using the CHO cells transfected with human FAPalpha described in Example 6.1. To confirm that significant bioactivity is only recruited by binding to membrane-proximal target epitopes of human FAPalpha - in addition - CHO cells expressing the murine FAPalpha antigen as described in
- 15 Example 6.3 and CHO cells expressing the mutated human FAPalpha antigen with murine membrane-distal epitopes as described in Example 6.4 are used. As effector cells stimulated human CD4/CD56 depleted PBMC are used.

Stimulated human PBMC are obtained as follows:

- A Petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) is coated
- 20 with a commercially available anti-CD3 specific antibody (e.g. OKT3, Orthoclone) in a final concentration of 1 $\mu\text{g}/\text{ml}$ for 1 hour at 37°C. Unbound protein is removed by one washing step with PBS. The fresh PBMC are isolated from peripheral blood (30 – 50 ml human blood) by Ficoll gradient centrifugation according to standard protocols. 3-5 x 10⁷ PBMC are added to the precoated petri dish in 120 ml of RPMI 1640 with
- 25 stabilized glutamine / 10% FCS / IL-2 20 U/ml (Proleukin, Chiron) and stimulated for 2 days. On the third day the cells are collected and washed once with RPMI 1640. IL 2 is added to a final concentration of 20 U/ml and the cells are cultivated again for one day in the same cell culture medium as above.

- By depletion of CD4+ T cells and CD56+ NK cells according to standard protocols
- 30 CD8+ cytotoxic T lymphocytes (CTLs) are enriched.

Target cells are washed twice with PBS and labeled with 11.1 MBq ^{51}Cr in a final volume of 100 μl RPMI with 50% FCS for 60 minutes at 37°C. Subsequently the labeled target cells are washed 3 times with 5 ml RPMI and then used in the cytotoxicity assay. The assay is performed in a 96 well plate in a total volume of

250µl supplemented RPMI (as above) with an E:T ratio of 10:1. Supernatant of cells expressing the bispecific single chain antibody molecules in a final concentration of 6,6% and 14 threefold dilutions thereof are applied. The assay time is 18 hours. Cytotoxicity is measured as relative values of released chromium in the supernatant
 5 related to the difference of maximum lysis (addition of Triton-X) and spontaneous lysis (without effector cells). All measurements are done in quadruplicates. Measurement of chromium activity in the supernatants is performed with a Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany). Analysis of the experimental data is performed with Prism 4 for Windows (version 4.02, GraphPad
 10 Software Inc., San Diego, California, USA). Sigmoidal dose response curves typically have R^2 values >0.90 as determined by the software.

Only those constructs showing potent recruitment of cytotoxic activity of effector T cells against target cells positive for FAPalpha are selected for further use. As shown in Figure 12 all of the generated bispecific antibodies directed at membrane-
 15 proximal target epitopes of human FAPalpha demonstrated cytotoxic activity against human FAPA positive target cells and target cells positive for the mutated human FAPalpha antigen with murine membrane-distal epitopes elicited by stimulated human CD4/CD56 depleted PBMC but did not recruit significant cytotoxic activity against murine FAPalpha positive target cells. Thereby specific recruitment of
 20 cytotoxic activity via binding to membrane-proximal target epitopes of human FAPalpha was confirmed.

6.15 Generation of CHO cells expressing macaque FAPalpha

The cDNA sequence of macaque FAPalpha is obtained by a set of four PCRs on cDNA from macaque monkey skin prepared according to standard protocols. The
 25 following reaction conditions: 1 cycle at 94°C for 3 minutes followed by 40 cycles with 94°C for 0.5 minutes, 56°C for 0.5 minutes and 72°C for 3 minutes followed by a terminal cycle of 72°C for 3 minutes and the following primers are used:

1. forward primer: 5'-cagcttccaactacaaagacagac-3' SEQ ID NO: 376
 reverse primer: 5'-tttctcttcataaaccagctctgg-3' SEQ ID NO: 377
- 30 2. forward primer: 5'-ttgaaacaaagaccaggagatccacc-3' SEQ ID NO: 378
 reverse primer: 5'-agatggcaagtaacacacttcttgc-3' SEQ ID NO: 379
3. forward primer: 5'-gaagaaacatctacagaattagcattgg-3' SEQ ID NO: 380
 reverse primer: 5'-cacatttgaaaagaccagttccagatgc-3' SEQ ID NO: 381

4. forward primer: 5'-agattacagctgtcagaaaattcatagaaatgg-3' SEQ ID NO: 382

reverse primer: 5'-atataagggttttcagattctgatacaggc-3' SEQ ID NO: 383

These PCRs generate four overlapping fragments, which are isolated and sequenced according to standard protocols using the PCR primers, and thereby provided the cDNA sequence coding macaque FAPalpha. To generate a construct for expression of macaque FAPalpha a cDNA fragment is obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 384 and 385). This construct contains the complete coding sequence of macaque FAPalpha followed by a stop codon. The gene synthesis fragment is also designed as to contain a Kozak site for eukaryotic expression of the construct and restriction sites at the beginning and the end of the fragment containing the cDNA. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end, are utilised in the following cloning procedures. The gene synthesis fragment is cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

6.16 Flow cytometric analysis of cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human FAPalpha

In order to test the cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human FAPalpha the capability of the constructs to bind to macaque FAPalpha and macaque CD3, respectively, is investigated by FACS analysis. For this purpose the macaque FAPalpha transfected CHO cells as described in example 6.15 and the macaque T cell line 4119LnPx (kindly provided by Prof Fickenscher, Hygiene Institute, Virology, Erlangen-Nuernberg; published in Knappe A, et al., and Fickenscher H., Blood 2000, 95, 3256-

61) are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with 50 µl of cell culture supernatant of transfected cells expressing the cross-species specific bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti His antibodies are detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

The cross-species specific binding of the single chain molecules listed above was clearly detectable as shown in Figure 11. In the FACS analysis all constructs showed binding to macaque CD3 and macaque FAPA compared to the negative control.

7. Generation of bispecific single chain antibodies directed at membrane-proximal target epitopes of human c-MET

7.1 Generation of CHO cells expressing human c-MET

The coding sequence of human c-MET as published in GenBank (Accession number NM_000245) is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the human c-MET protein and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 368 and 387). The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end, are utilized in the following cloning procedures. Internal restriction sites are removed by silent mutation of the coding sequence in the gene synthesis fragment. The gene synthesis fragment is cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A

Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

7.2 Generation of a soluble human c-MET fusion protein

The modified coding sequence of human c-MET as described in Example 7.1 is used for the construction of an artificial cDNA sequence encoding a soluble fusion protein of human c-MET and murine IgG1 Fc. To generate a construct for expression of the soluble human c-MET fusion protein a cDNA fragment is obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 388 and 389). The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the human c-MET protein from amino acid 1 to 932 corresponding to the signal peptide and extracellular domains of human c-MET, followed in frame by the coding sequence of an artificial Ser₁-Gly₄-Ser₁-linker, followed in frame by the coding sequence of the hinge region and Fc gamma portion of murine IgG1, followed in frame by the coding sequence of a 6 histidine tag and a stop codon. The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are all carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. After two passages

of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F – 68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively a clone

5 of the expression plasmid with sequence-verified nucleotide sequence is used for transfection and protein expression in the FreeStyle 293 Expression System (Invitrogen GmbH, Karlsruhe, Germany) according to the manufacturer's protocol. Supernatant containing the expressed protein is obtained, cells are removed by centrifugation and the supernatant is stored at -20°C.

10 For purification of the soluble human c-MET fusion protein a goat anti-mouse Fc affinity column is prepared according to standard protocols using a commercially available affinity purified goat anti-mouse IgG Fc fragment specific antibody with minimal cross-reaction to human, bovine and horse serum proteins (Jackson ImmunoResearch Europe Ltd.). Using this affinity column the fusion protein is

15 isolated out of cell culture supernatant on an Äkta Explorer System (GE Amersham) and eluted by citric acid. The eluate is neutralized and concentrated.

7.3 Generation of CHO cells expressing murine c-MET

The sequence of murine c-MET (NM_008591 Mus musculus met proto-oncogene (Met), mRNA, National Center for Biotechnology Information,

20 <http://www.ncbi.nlm.nih.gov/entrez>) is used to obtain a synthetic cDNA molecule by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the

25 complete coding sequence of the mature murine c-MET (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 390 and 391). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et

30 al. Cancer Immunol Immunother 50 (2001) 141-150) Internal restriction sites are removed by silent mutation of the coding sequence in the gene synthesis fragment. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with

sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

7.4 Generation of CHO cells expressing a mutated human c-MET antigen with murine membrane-distal epitopes

The coding sequence of a mutated human c-MET antigen with murine membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the coding sequence of the alpha-chain of the sema domain of mature murine c-MET followed in frame by human c-MET from the beta-chain of the sema domain to the stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 392 and 393). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). Internal restriction sites are removed by silent mutation of the coding sequence in the gene synthesis fragment. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

7.5 Generation of CHO cells expressing a mutated murine c-MET antigen with human membrane-distal epitopes

The coding sequence of a mutated murine c-MET antigen with human membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the coding sequence of the alpha-chain of the sema domain of mature human c-MET, followed in frame by murine c-MET from the beta-chain of the sema domain to the stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 394 and 395). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). Internal restriction sites are removed by silent mutation of the coding sequence in the gene synthesis fragment. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

7.6 Immunization of mice using a soluble human c-MET fusion protein

Twelve weeks old F1 mice from BALB/c x C57BL/6 crossings are immunized with the soluble human c-MET fusion protein as described in Example 7.2. To this end for each animal 40 µg of the soluble human c-MET fusion protein are mixed with 10 nmol of a thioate-modified CpG-Oligonucleotide (5'-tccatgacgttctctgatgct-3') in 300 µl PBS and are injected intraperitoneally. Mice receive booster immunizations after 21, 42 and optionally 63 days in the same way. Ten days after the first booster immunization, blood samples are taken and antibody serum titers against human c-MET are tested by flow cytometry according to standard protocols. To this end 200.000 cells of the human c-MET transfected CHO cells as described in Example

7.17 are incubated for 30 min on ice with 50 µl of serum of the immunized animals diluted 1:1000 in PBS with 2% FCS. The cells are washed twice in PBS with 2% FCS and binding of serum antibodies is detected with an mouse Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS.

5 Serum of the animals obtained prior to immunization is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, 10 Shevach and Strober, Wiley-Interscience, 2002).

Animals demonstrating significant serum reactivity against human c-MET as determined by the FACS analysis are used in the subsequent experiment.

7.7 Generation of an immune murine antibody scFv library: Construction of a combinatorial antibody library and phage display

15 Three days after the last booster immunization spleen cells of reactive animals are harvested for the preparation of total RNA according to standard protocols.

A library of murine immunoglobulin (Ig) light chain (kappa) variable region (VK) and Ig heavy chain variable region (VH) DNA-fragments is constructed by RT-PCR on murine spleen RNA using VK-and VH specific primers. cDNA is synthesized 20 according to standard protocols, see example 2.7.

450 ng of the kappa light chain fragments (SacI-SpeI digested) are ligated with 1400 ng of the phagemid pComb3H5BHis (SacI-SpeI digested; large fragment). The resulting combinatorial antibody library is then transformed into 300 µl of electrocompetent Escherichia coli XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm, Biorad gene-pulser) resulting in a library size of more 25 than 10^7 independent clones. After one hour of phenotype expression, positive transformants are selected for carbenicillin resistance encoded by the pComb3H5BHis vector in 100 ml of liquid super broth (SB)-culture over night. Cells are then harvested by centrifugation and plasmid preparation is carried out using a 30 commercially available plasmid preparation kit (Qiagen).

2800 ng of this plasmid-DNA containing the VK-library (XhoI-BstEII digested; large fragment) are ligated with 900 ng of the heavy chain V-fragments (XhoI-BstEII digested) and again transformed into two 300 µl aliquots of electrocompetent E. coli XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm)

resulting in a total VH-VK scFv (single chain variable fragment) library size of more than 10^7 independent clones.

After phenotype expression and slow adaptation to carbenicillin, the E. coli cells containing the antibody library are transferred into SB-Carbenicillin (SB with
5 50 µg/mL carbenicillin) selection medium. The E. coli cells containing the antibody library are then infected with an infectious dose of 10^{12} particles of helper phage VCSM13 resulting in the production and secretion of filamentous M13 phage, wherein each phage particle contains single stranded pComb3H5BHis-DNA encoding a murine scFv-fragment and displays the corresponding scFv-protein as a
10 translational fusion to phage coat protein III. This pool of phages displaying the antibody library is later used for the selection of antigen binding entities.

7.8 Phage display based selection of membrane-proximal target binders on CHO cells expressing the mutated human c-MET antigen with murine membrane-distal epitopes

15 The phage library carrying the cloned scFv-repertoire is harvested from the respective culture supernatant by PEG8000/NaCl precipitation and centrifugation. Approximately 10^{11} to 10^{12} scFv phage particles are resuspended in 0.4 ml of PBS/0.1% BSA and incubated with 10^5 to 10^7 CHO cells expressing the mutated human c-MET antigen with murine membrane-distal epitopes as described in
20 example 7.17 for 1 hour on ice under slow agitation. These CHO cells are grown beforehand, harvested by centrifugation, washed in PBS and resuspended in PBS/1 % FCS (containing Na Azide). scFv phage which do not specifically bind to the CHO cells are eliminated by up to five washing steps with PBS/1 % FCS (containing Na Azide). After washing, binding entities are eluted from the cells by resuspending the
25 cells in HCl-glycine pH 2.2 (10 min incubation with subsequent vortexing) and after neutralization with 2 M Tris pH 12, the eluate is used for infection of a fresh uninfected E. coli XL1 Blue culture ($OD_{600} > 0.5$). The E. coli culture containing E. coli cells successfully transduced with a phagemid copy, encoding a murine scFv-fragment, are again selected for carbenicillin resistance and subsequently infected
30 with VCMS 13 helper phage to start the second round of antibody display and in vitro selection. Typically a total of 4 to 5 rounds of selections are carried out.

7.9 Screening for membrane-proximal target binders on CHO cells expressing the human c-MET antigen, the murine c-MET antigen, the mutated human c-

MET antigen with murine membrane-distal epitopes and the mutated murine c-MET antigen with human membrane-distal epitopes

Plasmid DNA corresponding to 4 and 5 rounds of panning is isolated from E. coli cultures after selection. For the production of soluble scFv-protein, VH-VL-DNA fragments are excised from the plasmids (XhoI-SpeI). These fragments are cloned via the same restriction sites in the plasmid pComb3H5BFlag/His differing from the original pComb3H5BHis in that the expression construct (e.g. scFv) includes a Flag-tag (TGDKDDDDK) between the scFv and the His6-tag and the additional phage proteins are deleted. After ligation, each pool (different rounds of panning) of plasmid DNA is transformed into 100 µl heat shock competent E. coli TG1 or XLI blue and plated onto carbenicillin LB-agar. Single colonies are picked into 100 µl of LB carb (LB with 50 µg/ml carbenicillin).

After induction with 1 mM IPTG E. coli transformed with pComb3H5BFlag/His containing a VL-and VH-segment produce soluble scFv in sufficient amounts. Due to a suitable signal sequence, the scFv is exported into the periplasma where it folds into a functional conformation.

Single E. coli bacterial colonies from the transformation plates are picked for periplasmic small scale preparations and grown in SB-medium (e.g. 10 ml) supplemented with 20 mM MgCl₂ and carbenicillin 50µg/ml (and re-dissolved in PBS (e.g. 1 ml) after harvesting. A temperature shock is applied by four rounds of freezing at -70°C and thawing at 37°C whereby the outer membrane of the bacteria is destroyed and the soluble periplasmic proteins including the scFvs are released into the supernatant. After elimination of intact cells and cell-debris by centrifugation, the supernatant containing the murine anti-human c-MET-scFvs is collected and used for further examination.

Screening of the isolated scFvs for membrane-proximal target binders is performed by flow cytometry on CHO cells expressing the human c-MET antigen as described in Example 7.17, the murine c-MET antigen as described in Example 7.17, the mutated human c-MET antigen with murine membrane-distal epitopes as described in Example 7.17 and the mutated murine c-MET antigen with human membrane-distal epitopes as described in Example 7.17.

For flow cytometry 2.5×10^5 cells of the respective cell lines are incubated with 50 µl supernatant. The binding of the constructs is detected with an anti-His antibody (Penta-His Antibody, BSA free, Qiagen GmbH, Hilden, FRG) at 2 µg/ml in 50 µl PBS

with 2% FCS. As a second step reagent a R-Phycoerythrin-conjugated affinity purified F(ab')₂ fragment, goat anti-mouse IgG (Fc-gamma fragment specific), diluted 1:100 in 50 µl PBS with 2% FCS (Dianova, Hamburg, FRG) is used. The samples are measured on a FACSScan (BD biosciences, Heidelberg, FRG).

- 5 Only constructs which show binding to CHO cells expressing the human c-MET antigen and show binding to CHO cells expressing the mutated human c-MET antigen with murine membrane-distal epitopes and do not show binding to CHO cells expressing the murine c-MET antigen and also do not show binding to CHO cells expressing the mutated murine c-MET antigen with human membrane-distal epitopes
- 10 are selected for further use.

scFv specific for membrane proximal epitopes of human cMET were generated as described above and designated as set out in the following Table 6:

Table 6: Designation of single chain antibody fragments

SEQ ID (nucl/prot)	Designation
734/733	ME06F2HL
720/719	ME06E10HL
706/705	ME06D2HL
692/691	ME06D1HL
664/663	ME06C7HL
650/649	ME06C6HL
678/677	ME06B7HL
636/635	ME05F6HL
622/621	ME05B7HL
608/607	ME99B1HL
594/593	ME75H6HL

15

Membrane-proximal target binding of the single chain molecules listed above was clearly detectable as shown in Figure 16. In the FACS analysis all constructs showed binding to the human c-MET antigen and showed binding to the mutated human c-MET antigen with murine membrane-distal epitopes and did not show binding to the

20 murine c-MET antigen and did also not show binding to the mutated murine c-MET antigen with human membrane-distal epitopes as compared to the negative control.

7.10 Generation of human/humanized equivalents of non-human scFvs to membrane-proximal target epitopes of human c-MET

The VH region of a murine anti-c-MET scFv to a membrane-proximal target epitope of human c-MET is aligned against human antibody germline amino acid sequences.

- 5 The human antibody germline VH sequence is chosen which has the closest homology to the non-human VH and a direct alignment of the two amino acid sequences is performed. There are a number of framework residues of the non-human VH that differ from the human VH framework regions ("different framework positions"). Some of these residues may contribute to the binding and activity of the
- 10 antibody to its target.

To construct a library that contains the murine CDRs and at every framework position that differs from the chosen human VH sequence both possible residues (the human and the maternal murine amino acid residue), degenerated oligonucleotides are synthesized. These oligonucleotides incorporate at the differing positions the human

15 residue with a probability of 75 % and the murine residue with a probability of 25 %. For one human VH e.g. six of these oligonucleotides have to be synthesized that overlap in a terminal stretch of approximately 20 nucleotides. To this end every second primer is an antisense primer. Restriction sites within the oligonucleotides needed for later cloning are deleted.

- 20 These primers may have a length of 60 to 90 nucleotides, depending on the number of primers that are needed to span over the whole V sequence.

These e.g. six primers are mixed in equal amounts (e.g. 1 µl of each primer (primer stocks 20 to 100 µM) to a 20 µl PCR reaction) and added to a PCR mix consisting of PCR buffer, nucleotides and Taq polymerase. This mix is incubated at 94 °C for 3

25 minutes, 65 °C for 1 minute, 62°C for 1 minute, 59 °C for 1 minute, 56 °C for 1 minute, 52 °C for 1 minute, 50 °C for 1 minute and at 72°C for 10 minutes in a PCR cyclor. Subsequently the product is run in an agarose gel electrophoresis and the product of a size from 200 to 400 base pairs isolated from the gel according to standard methods.

- 30 This PCR product is then used as a template for a standard PCR reaction using primers that incorporate suitable N-terminal and C-terminal cloning restriction sites. The DNA fragment of the correct size (for a VH approximately 350 nucleotides) is isolated by agarose gel electrophoresis according to standard methods. In this way sufficient VH DNA fragment is amplified. This VH fragment is now a pool of VH

fragments that have each one a different amount of human and murine residues at the respective differing framework positions (pool of humanized VH). The same procedure is performed for the VL region of the murine anti-c-MET scFv to a membrane-proximal target epitope of human c-MET (pool of humanized VL).

- 5 The pool of humanized VH is then combined with the pool of humanized VL in the phage display vector pComb3H5Bhis to form a library of functional scFvs from which – after display on filamentous phage - anti-c-MET binders to membrane-proximal target epitopes of human c-MET are selected, screened, identified and confirmed as described above for the parental non-human (murine) anti-c-MET scFv. Single clones
- 10 are then analyzed for favorable properties and amino acid sequence. Those scFvs, which are closest in amino acid sequence homology to human germline V-segments, are preferred.

Human/humanized anti-c-MET scFvs to membrane-proximal target epitopes of human c-MET are converted into recombinant bispecific single chain antibodies and

15 further characterized as follows.

7.11 Generation of I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human c-MET

Anti-c-MET scFvs to membrane-proximal target epitopes of human c-MET with favorable properties and amino acid sequence are converted into recombinant

20 bispecific single chain antibodies by joining them via a Gly₄Ser₁-linker with the CD3 specific scFv I2C (SEQ ID NO: 185) to result in constructs with the domain arrangement VH_{c-MET} - (Gly₄Ser₁)₃ - VL_{c-MET} - Ser₁Gly₄Ser₁ - VH_{CD3} - (Gly₄Ser₁)₃ - VL_{CD3}.

I2C-based bispecific single chain antibodies directed at membrane-proximal target

25 epitopes of human c-MET were designed as set out in the following Table 7:

Table 7: Formats of I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human c-MET

SEQ ID (nucl/prot)	Formats of protein constructs (N → C)
512/511	ME86H11HLxI2CHL
526/525	ME62A12HLxI2CHL
540/539	ME63F2HLxI2CHL

554/553	ME62D11HLxI2CHL
568/567	ME62C10HLxI2CHL
582/581	ME62A4HLxI2CHL

Alternatively further constructs with different domain arrangements can be generated according to standard protocols. For expression in CHO cells the coding sequences of (i) an N-terminal immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence and (ii) a C-terminal His6-tag followed by a stop codon are both attached in frame to the nucleotide sequence encoding the bispecific single chain antibodies prior to insertion of the resulting DNA-fragment as obtained by gene synthesis into the multiple cloning site of the expression vector pEF-DHFR (Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

7.12 Expression and purification of bispecific single chain antibody molecules directed at membrane-proximal target epitopes of human c-MET

Bispecific single chain antibody molecules are expressed in Chinese hamster ovary cells (CHO). Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the constructs is induced by addition of increasing concentrations of MTX up to final concentrations of 20 nM MTX. After two passages of stationary culture cell culture supernatant is collected and used in the subsequent experiments. To generate supernatant for purification after two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F-68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively, constructs are transiently expressed in HEK 293 cells. Transfection is performed with 293fectin reagent (Invitrogen, #12347-019) according to the manufacturer's protocol. Furthermore the constructs are alternatively expressed in transiently transfected DHFR deficient CHO

cells using for example FuGENE® HD Transfection Reagent (Roche Diagnostics GmbH, Cat. No. 04709691001) according to the manufacturer's protocol.

Äkta® Explorer System (GE Health Systems) and Unicorn® Software are used for chromatography. Immobilized metal affinity chromatography ("IMAC") is performed using a Fractogel EMD chelate® (Merck) which is loaded with ZnCl₂ according to the protocol provided by the manufacturer. The column is equilibrated with buffer A (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl) and the cell culture supernatant (500 ml) is applied to the column (10 ml) at a flow rate of 3 ml/min. The column is washed with buffer A to remove unbound sample. Bound protein is eluted using a two step gradient of buffer B (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl, 0.5 M Imidazole) according to the following procedure:

Step 1: 20% buffer B in 6 column volumes

Step 2: 100% buffer B in 6 column volumes

Eluted protein fractions from step 2 are pooled for further purification. All chemicals are of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

Gel filtration chromatography is performed on a HiLoad 16/60 Superdex 200 prep grade column (GE/Amersham) equilibrated with Equi-buffer (25 mM Citrate, 200 mM Lysine, 5% Glycerol, pH 7.2). Eluted protein samples (flow rate 1 ml/min) are subjected to standard SDS-PAGE and Western Blot for detection. Prior to purification, the column is calibrated for molecular weight determination (molecular weight marker kit, Sigma MW GF-200). Protein concentrations are determined using OD280 nm.

Purified bispecific single chain antibody protein is analyzed in SDS PAGE under reducing conditions performed with pre-cast 4-12% Bis Tris gels (Invitrogen). Sample preparation and application are performed according to the protocol provided by the manufacturer. The molecular weight is determined with MultiMark protein standard (Invitrogen). The gel is stained with colloidal Coomassie (Invitrogen protocol). The purity of the isolated protein is typically >95% as determined by SDS-PAGE.

The bispecific single chain antibody has a molecular weight of about 52 kDa under native conditions as determined by gel filtration in PBS.

Western Blot is performed using an Optitrans® BA-S83 membrane and the Invitrogen Blot Module according to the protocol provided by the manufacturer. The antibody used is directed against the His Tag (Penta His, Qiagen) and a Goat-anti-mouse Ig

labeled with alkaline phosphatase (AP) (Sigma) is used as second step reagent, and BCIP/NBT (Sigma) as substrate. A band detected at 52 kD corresponds to purified bispecific single chain antibodies.

5 **7.13 Flow cytometric binding analysis of bispecific antibodies directed at membrane-proximal target epitopes of human c-MET**

In order to test the functionality of bispecific antibody constructs regarding the capability to bind to CD3 and to membrane-proximal target epitopes of human c-MET, respectively, a FACS analysis is performed. For this purpose CHO cells transfected with human c-MET as described in Example 7.17 and the human CD3
10 positive T cell leukemia cell line HPB-ALL (DSMZ, Braunschweig, ACC483) are used. For confirmation of binding to membrane-proximal target epitopes of human c-MET - in addition - CHO cells expressing the murine c-MET antigen as described in Example 7.17, CHO cells expressing the mutated human c-MET antigen with murine membrane-distal epitopes as described in Example 7.17 and CHO cells expressing
15 the mutated murine c-MET antigen with human membrane-distal epitopes as described in Example 7.17 are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with 50 µl of cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody
20 (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti His antibodies are detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is
25 used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Only those constructs that show bispecific binding to human CD3 as well as to
30 human c-MET and neither bind to the murine c-MET antigen nor to the mutated murine c-MET antigen with human membrane-distal epitopes are selected for further use.

The bispecific binding of the single chain molecules listed above was clearly detectable as shown in Figure 13. In the FACS analysis all constructs showed binding to human CD3 and human c-MET compared to the negative control.

Membrane-proximal target binding of the single chain molecules listed above was clearly detectable as shown in Figure 14. In the FACS analysis all constructs showed binding to the mutated human c-MET antigen with murine membrane-distal epitopes and did not show binding to the murine c-MET antigen and did also not show binding to the mutated murine c-MET antigen with human membrane-distal epitopes as compared to the negative control. Expression of the c-MET antigens was confirmed by detection with an anti-FLAG M2 antibody as described herein. In the FACS analysis also shown in Figure 14 CHO cells transfected with the murine c-MET antigen, the mutated murine c-MET antigen with human membrane-distal epitopes and the mutated human c-MET antigen with murine membrane-distal epitopes, respectively, showed comparable expression of the antigens as detected with the anti-FLAG antibody.

7.14 Bioactivity of bispecific antibodies directed at membrane-proximal target epitopes of human c-MET

Bioactivity of generated bispecific single chain antibodies is analyzed by chromium 51 (^{51}Cr) release in vitro cytotoxicity assays using the CHO cells transfected with human c-MET described in Example 7.17. To confirm that significant bioactivity is only recruited by binding to membrane-proximal target epitopes of human c-MET - in addition - CHO cells expressing murine c-MET and the mutated murine c-MET antigen with human membrane-distal epitopes, respectively, both as described in Example 7.17 are used. As effector cells stimulated human CD4/CD56 depleted PBMC are used.

Stimulated human PBMC are obtained as follows:

A Petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) is coated with a commercially available anti-CD3 specific antibody (e.g. OKT3, Orthoclone) in a final concentration of 1 $\mu\text{g/ml}$ for 1 hour at 37°C. Unbound protein is removed by one washing step with PBS. The fresh PBMC are isolated from peripheral blood (30 – 50 ml human blood) by Ficoll gradient centrifugation according to standard protocols. 3 - 5 x 10⁷ PBMC are added to the precoated petri dish in 120 ml of RPMI 1640 with stabilized glutamine / 10% FCS / IL-2 20 U/ml (Proleukin, Chiron) and stimulated for 2 days. On the third day the cells are collected and washed once with RPMI 1640. IL

2 is added to a final concentration of 20 U/ml and the cells are cultivated again for one day in the same cell culture medium as above.

By depletion of CD4+ T cells and CD56+ NK cells according to standard protocols CD8+ cytotoxic T lymphocytes (CTLs) are enriched.

- 5 Target cells are washed twice with PBS and labeled with 11.1 MBq ^{51}Cr in a final volume of 100 μl RPMI with 50% FCS for 60 minutes at 37°C. Subsequently the labeled target cells are washed 3 times with 5 ml RPMI and then used in the cytotoxicity assay. The assay is performed in a 96 well plate in a total volume of 250 μl supplemented RPMI (as above) with an E:T ratio of 10:1. Supernatant of cells
- 10 expressing the bispecific single chain antibody molecules in a final concentration of 50% and 20 threefold dilutions thereof are applied. The assay time is 18 hours. Cytotoxicity is measured as relative values of released chromium in the supernatant related to the difference of maximum lysis (addition of Triton-X) and spontaneous lysis (without effector cells). All measurements are done in quadruplicates.
- 15 Measurement of chromium activity in the supernatants is performed with a Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany). Analysis of the experimental data is performed with Prism 4 for Windows (version 4.02, GraphPad Software Inc., San Diego, California, USA). Sigmoidal dose response curves typically have R^2 values >0.90 as determined by the software.
- 20 Only those constructs showing potent recruitment of cytotoxic activity of effector T cells against target cells positive for c-MET are selected for further use.

As shown in Figure 15 all of the generated bispecific antibodies directed at membrane-proximal target epitopes of human c-MET demonstrated cytotoxic activity against human c-MET positive target cells elicited by stimulated human CD4/CD56

25 depleted PBMC but did not recruit significant cytotoxic activity against murine c-MET positive target cells and target cells positive for the mutated murine c-MET antigen with human membrane-distal epitopes. Thereby specific recruitment of cytotoxic activity via binding to membrane-proximal target epitopes of human c-MET was confirmed.

30 **7.15 Generation of CHO cells expressing macaque c-MET**

The cDNA sequence of macaque c-MET is obtained by a set of 5 PCRs on cDNA from macaque monkey Liver prepared according to standard protocols. The following reaction conditions: 1 cycle at 94°C for 2 minutes followed by 40 cycles with 94°C for

1 minute, 56°C for 1 minute and 72°C for 3 minutes followed by a terminal cycle of 72°C for 3 minutes and the following primers are used:

1. forward primer: 5'-aggaattcaccatgaaggcccccgcgtgtgcttgccacc-3' SEQ ID NO: 396
reverse primer: 5'-ctccagaggcatttccatgtagg-3' SEQ ID NO: 397
- 5 2. forward primer: 5'-gtccaaagggaactctagatgc-3' SEQ ID NO: 398
reverse primer: 5'-ggagacactggatgggagtcagg-3' SEQ ID NO: 399
3. forward primer: 5'-catcagagggcgcttcatgcagg-3' SEQ ID NO: 400
reverse primer: 5'-gctttggttttcagggggagttgc-3' SEQ ID NO: 401
4. forward primer: 5'-atccaaccaaattctttattagtgtgg-3' SEQ ID NO: 402
10 reverse primer: 5'-gacttcattgaaatgcacaatcagg-3' SEQ ID NO: 403
5. forward primer: 5'-tgctctaaatccagagctgtcc-3' SEQ ID NO: 404
reverse primer: 5'-gtcagataagaaattccttagaatcc-3' SEQ ID NO: 405

These PCRs generate five overlapping fragments, which are isolated and sequenced according to standard protocols using the PCR primers, and thereby provide a

15 portion of the cDNA sequence coding macaque c-MET from codon 10 of the leader peptide to the last codon of the mature protein. To generate a construct for expression of macaque c-MET a cDNA fragment is obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 406 and 407). In this construct the coding sequence of

20 macaque c-MET from amino acid 10 of the leader peptide to the last amino acid of the mature c-MET protein followed by a stop codon is fused in frame to the coding sequence of the amino acids 1 to 9 of the leader peptide of the human c-MET protein. The gene synthesis fragment is also designed as to contain a Kozak site for eukaryotic expression of the construct and restriction sites at the beginning and the

25 end of the fragment containing the cDNA. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end, are utilised in the following cloning procedures. Internal restriction sites are removed by silent mutation of the coding sequence in the gene synthesis fragment. The gene synthesis fragment is cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer

30 Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells

for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

5 **7.16 Flow cytometric analysis of cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human c-MET**

In order to test the cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human c-MET the capability of the constructs to bind to macaque c-MET and macaque CD3, respectively, is investigated by FACS
10 analysis. For this purpose the macaque c-MET transfected CHO cells as described in example 7.17 and the macaque T cell line 4119LnPx (kindly provided by Prof Fickenscher, Hygiene Institute, Virology, Erlangen-Nuernberg; published in Knappe A, et al., and Fickenscher H., Blood 2000, 95, 3256-61) are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with with 50 µl of cell culture
15 supernatant of transfected cells expressing the cross-species specific bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti His antibodies are detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in
20 PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control. Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies,
25 Shevach and Strober, Wiley-Interscience, 2002).

The cross-species specific binding of the single chain molecules listed above was clearly detectable as shown in Figure 13. In the FACS analysis all constructs showed binding to macaque CD3 and macaque c-MET compared to the negative control.

30 **7.17 Generation of CHO cells with enhanced expression of extracellular domains of human c-MET, macaque c-MET, murine c-MET, mutated murine c-MET with human membrane-distal epitopes and mutated human c-MET with murine membrane-distal epitopes, respectively**

The modified coding sequences of human c-MET, macaque c-MET, murine c-MET, mutated murine c-MET with human membrane-distal epitopes and mutated human c-

MET with murine membrane-distal epitopes as described above are used for the construction of artificial cDNA sequences encoding fusion proteins of the extracellular domains of human c-MET, macaque c-MET, murine c-MET, mutated murine c-MET with human membrane-distal epitopes and mutated human c-MET with murine membrane-distal epitopes, respectively, with a truncated variant of human EpCAM. To generate constructs for expression of these c-MET fusion proteins cDNA fragments are obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequences of the constructs are listed under SEQ ID Nos 489 and 490 for human c-MET, 491 and 492 for macaque c-MET, 493 and 494 for murine c-MET, 495 and 496 for mutated murine c-MET with human membrane-distal epitopes and 497 and 498 for mutated human c-MET with murine membrane-distal epitopes). The gene synthesis fragments are designed as to contain first a Kozak site for eukaryotic expression of the construct, followed by the coding sequence of a 19 amino acid immunoglobulin leader peptide, followed in frame by the coding sequence of a FLAG tag (only in the case of the murine and the mutated human and mutated murine constructs), followed in frame by the coding sequence of the extracellular domains of human c-MET, macaque c-MET, murine c-MET, mutated murine c-MET with human membrane-distal epitopes and mutated human c-MET with murine membrane-distal epitopes, respectively, followed in frame by the coding sequence of an artificial Ser₁-Gly₄-Ser₁-Gly₁-linker, followed in frame by the coding sequence of the transmembrane domain and intracellular domain of human EpCAM (as published in GenBank; Accession number NM_002354; amino acids 266 to 314 [as counted from the start codon] except for a point mutation at position 279 with isoleucine instead of valine) and a stop codon. The gene synthesis fragments are also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragments are cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. Clones with sequence-verified nucleotide sequence are transfected into DHFR deficient CHO cells for eukaryotic expression of the constructs. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the constructs is

induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

8. Generation of bispecific single chain antibodies directed at membrane-proximal target epitopes of human Endosialin

5 8.1 Generation of CHO cells expressing human Endosialin

The coding sequence of human Endosialin as published in GenBank (Accession number NM_020404) is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the human Endosialin protein, followed in frame by the coding sequence of a Flag tag and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 408 and 409). The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, EcoRI at the 5' end and XbaI at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via EcoRI and XbaI into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

8.2 Generation of a soluble human Endosialin fusion protein

The coding sequence of human Endosialin as described in Example 8.1 is used for the construction of an artificial cDNA sequence encoding a soluble fusion protein of human Endosialin and murine IgG1 Fc. To generate a construct for expression of the soluble human Endosialin fusion protein a cDNA fragment is obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 410 and 411). The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct

followed by the coding sequence of the human Endosialin protein from amino acid 1 to 685 corresponding to the signal peptide and extracellular domains of human Endosialin, followed in frame by the coding sequence of an artificial Thr₁-Gly₄-Ser₁-linker, followed in frame by the coding sequence of the hinge region and Fc gamma

5 portion of murine IgG1, followed in frame by the coding sequence of a 6 histidine tag and a stop codon. The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, EcoRI at the 5' end and XbaI at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via EcoRI and

10 XbaI into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are all carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with

15 sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

20 After two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F – 68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively a clone of the expression plasmid with sequence-verified

25 nucleotide sequence is used for transfection and protein expression in the FreeStyle 293 Expression System (Invitrogen GmbH, Karlsruhe, Germany) according to the manufacturer's protocol. Supernatant containing the expressed protein is obtained, cells are removed by centrifugation and the supernatant is stored at -20°C.

For purification of the soluble human Endosialin fusion protein a goat anti-mouse Fc

30 affinity column is prepared according to standard protocols using a commercially available affinity purified goat anti-mouse IgG Fc fragment specific antibody with minimal cross-reaction to human, bovine and horse serum proteins (Jackson ImmunoResearch Europe Ltd.). Using this affinity column the fusion protein is

isolated out of cell culture supernatant on an Äkta Explorer System (GE Amersham) and eluted by citric acid. The eluate is neutralized and concentrated.

8.3 Generation of CHO cells expressing the murine Endosialin antigen

The sequence of murine Endosialin (NM_054042 Mus musculus Endosialin antigen, endosialin (Cd248), mRNA; National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/entrez>) is used to obtain a synthetic cDNA molecule by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the complete coding sequence of mature murine endosialin (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 412 and 413). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

8.4 Generation of CHO cells expressing a mutated human Endosialin antigen with murine membrane-distal epitopes

The coding sequence of a mutated human Endosialin antigen with murine membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the coding sequence of the C-type lectin domain of mature murine endosialin followed in frame by human endosialin

from the Sushi/SCR/CCP domain to the stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 414 and 415). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

8.5 Generation of CHO cells expressing a mutated murine Endosialin antigen with human membrane-distal epitopes

The coding sequence of a mutated murine Endosialin antigen with human membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the coding sequence of the C-type lectin domain of mature human endosialin followed in frame by murine endosialin from the Sushi/SCR/CCP domain to the stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 416 and 417). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). Internal restriction sites are removed by silent mutation of the coding sequence in the gene synthesis fragment. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells

for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

8.6 Immunization of mice using a soluble human Endosialin fusion protein

Twelve weeks old F1 mice from BALB/c x C57BL/6 crossings are immunized with the soluble human Endosialin fusion protein as described in Example 8.2. To this end for each animal 40 µg of the soluble human Endosialin fusion protein are mixed with 10 nmol of a thioate-modified CpG-Oligonucleotide (5'-tccatgacgttctctgatgct-3') in 300 µl PBS and are injected intraperitoneally. Mice receive booster immunizations after 21, 42 and optionally 63 days in the same way. Ten days after the first booster immunization, blood samples are taken and antibody serum titers against human Endosialin are tested by flow cytometry according to standard protocols. To this end 200.000 cells of the human Endosialin transfected CHO cells as described in Example 8.1 are incubated for 30 min on ice with 50 µl of serum of the immunized animals diluted 1:1000 in PBS with 2% FCS. The cells are washed twice in PBS with 2% FCS and binding of serum antibodies is detected with an mouse Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Serum of the animals obtained prior to immunization is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Animals demonstrating significant serum reactivity against human Endosialin as determined by the FACS analysis are used in the subsequent experiment.

8.7 Generation of an immune murine antibody scFv library: Construction of a combinatorial antibody library and phage display

Three days after the last booster immunization spleen cells of reactive animals are harvested for the preparation of total RNA according to standard protocols.

A library of murine immunoglobulin (Ig) light chain (kappa) variable region (VK) and Ig heavy chain variable region (VH) DNA-fragments is constructed by RT-PCR on murine spleen RNA using VK-and VH specific primers. cDNA is synthesized according to standard protocols, see example 2.7.

5 450 ng of the kappa light chain fragments (SacI-SpeI digested) are ligated with 1400 ng of the phagemid pComb3H5BHis (SacI-SpeI digested; large fragment). The resulting combinatorial antibody library is then transformed into 300 µl of electrocompetent *Escherichia coli* XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm, Biorad gene-pulser) resulting in a library size of more
10 than 10^7 independent clones. After one hour of phenotype expression, positive transformants are selected for carbenicillin resistance encoded by the pComb3H5BHis vector in 100 ml of liquid super broth (SB)-culture over night. Cells are then harvested by centrifugation and plasmid preparation is carried out using a commercially available plasmid preparation kit (Qiagen).

15 2800 ng of this plasmid-DNA containing the VK-library (XhoI-BstEII digested; large fragment) are ligated with 900 ng of the heavy chain V-fragments (XhoI-BstEII digested) and again transformed into two 300 µl aliquots of electrocompetent *E. coli* XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm) resulting in a total VH-VK scFv (single chain variable fragment) library size of more
20 than 10^7 independent clones.

After phenotype expression and slow adaptation to carbenicillin, the *E. coli* cells containing the antibody library are transferred into SB-Carbenicillin (SB with 50 µg/mL carbenicillin) selection medium. The *E. coli* cells containing the antibody library are then infected with an infectious dose of 10^{12} particles of helper phage
25 VCSM13 resulting in the production and secretion of filamentous M13 phage, wherein each phage particle contains single stranded pComb3H5BHis-DNA encoding a murine scFv-fragment and displays the corresponding scFv-protein as a translational fusion to phage coat protein III. This pool of phages displaying the antibody library is later used for the selection of antigen binding entities.

30 **8.8 Phage display based selection of membrane-proximal target binders on CHO cells expressing the mutated human Endosialin antigen with murine membrane-distal epitopes**

The phage library carrying the cloned scFv-repertoire is harvested from the respective culture supernatant by PEG8000/NaCl precipitation and centrifugation.

Approximately 10^{11} to 10^{12} scFv phage particles are resuspended in 0.4 ml of PBS/0.1% BSA and incubated with 10^5 to 10^7 CHO cells expressing the mutated human Endosialin antigen with murine membrane-distal epitopes as described in example 8.4 for 1 hour on ice under slow agitation. These CHO cells are grown
5 beforehand, harvested by centrifugation, washed in PBS and resuspended in PBS/1 % FCS (containing Na Azide). scFv phage which do not specifically bind to the CHO cells are eliminated by up to five washing steps with PBS/1 % FCS (containing Na Azide). After washing, binding entities are eluted from the cells by resuspending the cells in HCl-glycine pH 2.2 (10 min incubation with subsequent vortexing) and after
10 neutralization with 2 M Tris pH 12, the eluate is used for infection of a fresh uninfected E. coli XL1 Blue culture ($OD_{600} > 0.5$). The E. coli culture containing E. coli cells successfully transduced with a phagemid copy, encoding a murine scFv-fragment, are again selected for carbenicillin resistance and subsequently infected with VCMS 13 helper phage to start the second round of antibody display and in vitro
15 selection. Typically a total of 4 to 5 rounds of selections are carried out.

8.9 Screening for membrane-proximal target binders on CHO cells expressing the human Endosialin antigen, the murine Endosialin antigen and the mutated murine Endosialin antigen with human membrane-distal epitopes

Plasmid DNA corresponding to 4 and 5 rounds of panning is isolated from E. coli
20 cultures after selection. For the production of soluble scFv-protein, VH-VL-DNA fragments are excised from the plasmids (XhoI-SpeI). These fragments are cloned via the same restriction sites in the plasmid pComb3H5BFlag/His differing from the original pComb3H5BHis in that the expression construct (e.g. scFv) includes a Flag-tag (TG DYKDDDDK) between the scFv and the His6-tag and the additional phage
25 proteins are deleted. After ligation, each pool (different rounds of panning) of plasmid DNA is transformed into 100 μ l heat shock competent E. coli TG1 or XLI blue and plated onto carbenicillin LB-agar. Single colonies are picked into 100 μ l of LB carb (LB with 50 μ g/ml carbenicillin).

After induction with 1 mM IPTG E. coli transformed with pComb3H5BFlag/His
30 containing a VL-and VH-segment produce soluble scFv in sufficient amounts. Due to a suitable signal sequence, the scFv is exported into the periplasma where it folds into a functional conformation.

Single E. coli bacterial colonies from the transformation plates are picked for periplasmic small scale preparations and grown in SB-medium (e.g. 10 ml)

supplemented with 20 mM MgCl₂ and carbenicillin 50µg/ml (and re-dissolved in PBS (e.g. 1 ml) after harvesting. A temperature shock is applied by four rounds of freezing at -70°C and thawing at 37°C whereby the outer membrane of the bacteria is destroyed and the soluble periplasmic proteins including the scFvs are released into the supernatant. After elimination of intact cells and cell-debris by centrifugation, the supernatant containing the murine anti-human Endosialin-scFvs is collected and used for further examination.

Screening of the isolated scFvs for membrane-proximal target binders is performed by flow cytometry on CHO cells expressing the human Endosialin antigen as described in Example 8.1, the murine Endosialin antigen as described in Example 8.3 and the mutated murine Endosialin antigen with human membrane-distal epitopes as described in Example 8.5.

For flow cytometry 2.5x10⁵ cells of the respective cell lines are incubated with 50 µl supernatant. The binding of the constructs is detected with an anti-His antibody (Penta-His Antibody, BSA free, Qiagen GmbH, Hilden, FRG) at 2 µg/ml in 50 µl PBS with 2% FCS. As a second step reagent a R-Phycoerythrin-conjugated affinity purified F(ab')₂ fragment, goat anti-mouse IgG (Fc-gamma fragment specific), diluted 1:100 in 50 µl PBS with 2% FCS (Dianova, Hamburg, FRG) is used. The samples are measured on a FACSScan (BD biosciences, Heidelberg, FRG).

Only constructs which show binding to CHO cells expressing the human Endosialin antigen and do not show binding to CHO cells expressing the murine Endosialin antigen and also do not show binding to CHO cells expressing the mutated murine Endosialin antigen with human membrane-distal epitopes are selected for further use.

8.10 Generation of human/humanized equivalents of non-human scFvs to membrane-proximal target epitopes of human Endosialin

The VH region of a murine anti-Endosialin scFv to a membrane-proximal target epitope of human Endosialin is aligned against human antibody germline amino acid sequences. The human antibody germline VH sequence is chosen which has the closest homology to the non-human VH and a direct alignment of the two amino acid sequences is performed. There are a number of framework residues of the non-human VH that differ from the human VH framework regions ("different framework positions"). Some of these residues may contribute to the binding and activity of the antibody to its target.

To construct a library that contains the murine CDRs and at every framework position that differs from the chosen human VH sequence both possible residues (the human and the maternal murine amino acid residue), degenerated oligonucleotides are synthesized. These oligonucleotides incorporate at the differing positions the human
5 residue with a probability of 75 % and the murine residue with a probability of 25 %. For one human VH e.g. six of these oligonucleotides have to be synthesized that overlap in a terminal stretch of approximately 20 nucleotides. To this end every second primer is an antisense primer. Restriction sites within the oligonucleotides needed for later cloning are deleted.

- 10 These primers may have a length of 60 to 90 nucleotides, depending on the number of primers that are needed to span over the whole V sequence.

These e.g. six primers are mixed in equal amounts (e.g. 1 µl of each primer (primer stocks 20 to 100 µM) to a 20 µl PCR reaction) and added to a PCR mix consisting of PCR buffer, nucleotides and Taq polymerase. This mix is incubated at 94 °C for 3
15 minutes, 65 °C for 1 minute, 62°C for 1 minute, 59 °C for 1 minute, 56 °C for 1 minute, 52 °C for 1 minute, 50 °C for 1 minute and at 72°C for 10 minutes in a PCR cyclor. Subsequently the product is run in an agarose gel electrophoresis and the product of a size from 200 to 400 base pairs isolated from the gel according to standard methods.

- 20 This PCR product is then used as a template for a standard PCR reaction using primers that incorporate suitable N-terminal and C-terminal cloning restriction sites. The DNA fragment of the correct size (for a VH approximately 350 nucleotides) is isolated by agarose gel electrophoresis according to standard methods. In this way sufficient VH DNA fragment is amplified. This VH fragment is now a pool of VH
25 fragments that have each one a different amount of human and murine residues at the respective differing framework positions (pool of humanized VH). The same procedure is performed for the VL region of the murine anti-Endosialin scFv to a membrane-proximal target epitope of human Endosialin (pool of humanized VL).

The pool of humanized VH is then combined with the pool of humanized VL in the
30 phage display vector pComb3H5Bhis to form a library of functional scFvs from which – after display on filamentous phage - anti-Endosialin binders to membrane-proximal target epitopes of human Endosialin are selected, screened, identified and confirmed as described above for the parental non-human (murine) anti-Endosialin scFv. Single clones are then analyzed for favorable properties and amino acid sequence. Those

scFvs, which are closest in amino acid sequence homology to human germline V-segments, are preferred.

Human/humanized anti-Endosialin scFvs to membrane-proximal target epitopes of human Endosialin are converted into recombinant bispecific single chain antibodies and further characterized as follows.

8.11 Generation of I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human Endosialin

Anti-Endosialin scFvs to membrane-proximal target epitopes of human Endosialin with favorable properties and amino acid sequence are converted into recombinant bispecific single chain antibodies by joining them via a Gly₄Ser₁-linker with the CD3 specific scFv I2C (SEQ ID NO: 185) to result in constructs with the domain arrangement VH_{Endosialin} - (Gly₄Ser₁)₃ - VL_{Endosialin} - Ser₁Gly₄Ser₁ - VH_{CD3} - (Gly₄Ser₁)₃ - VL_{CD3}. Alternatively further constructs with different domain arrangements can be generated according to standard protocols. For expression in CHO cells the coding sequences of (i) an N-terminal immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence and (ii) a C-terminal His₆-tag followed by a stop codon are both attached in frame to the nucleotide sequence encoding the bispecific single chain antibodies prior to insertion of the resulting DNA-fragment as obtained by gene synthesis into the multiple cloning site of the expression vector pEF-DHFR (Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

8.12 Expression and purification of bispecific single chain antibody molecules directed at membrane-proximal target epitopes of human Endosialin

Bispecific single chain antibody molecules are expressed in Chinese hamster ovary cells (CHO). Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the constructs is induced by addition of increasing concentrations of MTX up to final concentrations of 20 nM MTX. After two passages of stationary

culture cell culture supernatant is collected and used in the subsequent experiments. To generate supernatant for purification after two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F-68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively, constructs are transiently expressed in HEK 293 cells. Transfection is performed with 293fectin reagent (Invitrogen, #12347-019) according to the manufacturer's protocol. Furthermore the constructs are alternatively expressed in transiently transfected DHFR deficient CHO cells using for example FuGENE® HD Transfection Reagent (Roche Diagnostics GmbH, Cat. No. 04709691001) according to the manufacturer's protocol.

Äkta® Explorer System (GE Health Systems) and Unicorn® Software are used for chromatography. Immobilized metal affinity chromatography ("IMAC") is performed using a Fractogel EMD chelate® (Merck) which is loaded with ZnCl₂ according to the protocol provided by the manufacturer. The column is equilibrated with buffer A (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl) and the cell culture supernatant (500 ml) is applied to the column (10 ml) at a flow rate of 3 ml/min. The column is washed with buffer A to remove unbound sample. Bound protein is eluted using a two step gradient of buffer B (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl, 0.5 M Imidazole) according to the following procedure:

Step 1: 20% buffer B in 6 column volumes

Step 2: 100% buffer B in 6 column volumes

Eluted protein fractions from step 2 are pooled for further purification. All chemicals are of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

Gel filtration chromatography is performed on a HiLoad 16/60 Superdex 200 prep grade column (GE/Amersham) equilibrated with Equi-buffer (25 mM Citrate, 200 mM Lysine, 5% Glycerol, pH 7.2). Eluted protein samples (flow rate 1 ml/min) are subjected to standard SDS-PAGE and Western Blot for detection. Prior to purification, the column is calibrated for molecular weight determination (molecular weight marker kit, Sigma MW GF-200). Protein concentrations are determined using OD280 nm.

Purified bispecific single chain antibody protein is analyzed in SDS PAGE under reducing conditions performed with pre-cast 4-12% Bis Tris gels (Invitrogen). Sample

preparation and application are performed according to the protocol provided by the manufacturer. The molecular weight is determined with MultiMark protein standard (Invitrogen). The gel is stained with colloidal Coomassie (Invitrogen protocol). The purity of the isolated protein is typically >95% as determined by SDS-PAGE.

- 5 The bispecific single chain antibody has a molecular weight of about 52 kDa under native conditions as determined by gel filtration in PBS.

Western Blot is performed using an Optitran® BA-S83 membrane and the Invitrogen Blot Module according to the protocol provided by the manufacturer. The antibody used is directed against the His Tag (Penta His, Qiagen) and a Goat-anti-mouse Ig
10 labeled with alkaline phosphatase (AP) (Sigma) is used as second step reagent, and BCIP/NBT (Sigma) as substrate. A band detected at 52 kD corresponds to purified bispecific single chain antibodies.

8.13 Flow cytometric binding analysis of bispecific antibodies directed at membrane-proximal target epitopes of human Endosialin

- 15 In order to test the functionality of bispecific antibody constructs regarding the capability to bind to CD3 and to membrane-proximal target epitopes of human Endosialin, respectively, a FACS analysis is performed. For this purpose CHO cells transfected with human Endosialin as described in Example 8.1 and the human CD3 positive T cell leukemia cell line HPB-ALL (DSMZ, Braunschweig, ACC483) are
20 used. For confirmation of binding to membrane-proximal target epitopes of human Endosialin - in addition - CHO cells expressing the murine Endosialin antigen as described in Example 8.3 and CHO cells expressing the mutated murine Endosialin antigen with human membrane-distal epitopes as described in Example 8.5 are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with 50 µl of
25 cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti His antibodies are detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in
30 PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control. Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as

described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Only those constructs that show bispecific binding to human CD3 as well as to human Endosialin and neither bind to the murine Endosialin antigen nor to the mutated murine Endosialin antigen with human membrane-distal epitopes are selected for further use.

8.14 Bioactivity of bispecific antibodies directed at membrane-proximal target epitopes of human Endosialin

Bioactivity of generated bispecific single chain antibodies is analyzed by chromium 51 (^{51}Cr) release in vitro cytotoxicity assays using the CHO cells transfected with human Endosialin described in Example 8.1. As effector cells stimulated human CD4/CD56 depleted PBMC are used.

Stimulated human PBMC are obtained as follows:

A Petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) is coated with a commercially available anti-CD3 specific antibody (e.g. OKT3, Orthoclone) in a final concentration of 1 $\mu\text{g}/\text{ml}$ for 1 hour at 37°C. Unbound protein is removed by one washing step with PBS. The fresh PBMC are isolated from peripheral blood (30 – 50 ml human blood) by Ficoll gradient centrifugation according to standard protocols. 3 - 5 x 10⁷ PBMC are added to the precoated petri dish in 120 ml of RPMI 1640 with stabilized glutamine / 10% FCS / IL-2 20 U/ml (Proleukin, Chiron) and stimulated for 2 days. On the third day the cells are collected and washed once with RPMI 1640. IL 2 is added to a final concentration of 20 U/ml and the cells are cultivated again for one day in the same cell culture medium as above.

By depletion of CD4+ T cells and CD56+ NK cells according to standard protocols CD8+ cytotoxic T lymphocytes (CTLs) are enriched.

Target cells are washed twice with PBS and labeled with 11.1 MBq ^{51}Cr in a final volume of 100 μl RPMI with 50% FCS for 60 minutes at 37°C. Subsequently the labeled target cells are washed 3 times with 5 ml RPMI and then used in the cytotoxicity assay. The assay is performed in a 96 well plate in a total volume of 250 μl supplemented RPMI (as above) with an E:T ratio of 10:1. 1 $\mu\text{g}/\text{ml}$ of purified bispecific single chain antibody molecule and 20 threefold dilutions thereof are applied. The assay time is 18 hours. Cytotoxicity is measured as relative values of released chromium in the supernatant related to the difference of maximum lysis (addition of Triton-X) and spontaneous lysis (without effector cells). All

measurements are done in quadruplicates. Measurement of chromium activity in the supernatants is performed with a Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany). Analysis of the experimental data is performed with Prism 4 for Windows (version 4.02, GraphPad Software Inc., San Diego, California, USA). Sigmoidal dose response curves typically have R^2 values >0.90 as determined by the software. EC50 values calculated by the analysis program are used for comparison of bioactivity.

Only those constructs showing potent recruitment of cytotoxic activity of effector T cells against target cells positive for Endosialin are selected for further use.

10 **8.15 Generation of CHO cells expressing macaque Endosialin**

The cDNA sequence of macaque Endosialin is obtained by a set of 2 PCRs on cDNA from macaque monkey colon prepared according to standard protocols. The following reaction conditions: 1 cycle at 95°C for 5 minutes followed by 40 cycles with 95°C for 45 seconds, 50°C for 45 seconds and 72°C for 2 minutes followed by a terminal cycle of 72°C for 5 minutes and the following primers are used for the first PCR:

forward primer: 5'-atatgaattcgccaccatgctgctgcgcctgttgctggcc-3' SEQ ID NO: 418

reverse primer: 5'-gtcttcacatcttctcatcctcccc-3' SEQ ID NO: 419

20 The following reaction conditions: 1 cycle at 95°C for 5 minutes followed by 40 cycles with 95°C for 45 seconds, 58°C for 45 seconds and 72°C for 2 minutes followed by a terminal cycle of 72°C for 5 minutes and the following primers are used for the second PCR:

forward primer: 5'-gtcaactacgttggtggcttcgagtg-3' SEQ ID NO: 420

25 reverse primer: 5'-ggctagatcacttatcgatcatcatctttagtccacgctggttctgcaggtctgc-3' SEQ ID NO: 421

The PCR reactions are performed under addition of PCR grade betain to a final concentration of 1M. Those PCRs generate two overlapping fragments, which are isolated and sequenced according to standard protocols using the PCR primers, and thereby provided a portion of the cDNA sequence coding macaque Endosialin from codon 9 of the leader peptide to codon 733 of the mature protein. To generate a construct for expression of macaque Endosialin a cDNA fragment is obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 422 and 423). In this construct the coding

sequence of macaque Endosialin from amino acid 9 of the leader peptide to amino acid 733 of the mature protein, followed in frame by the coding sequence of amino acid 734 to the last amino acid of the mature human Endosialin protein, followed in frame by the coding sequence of a FLAG tag and a stop codon is fused in frame to the coding sequence of the amino acids 1 to 8 of the leader peptide of the human Endosialin protein. The gene synthesis fragment is also designed as to contain a Kozak site for eukaryotic expression of the construct and restriction sites at the beginning and the end of the fragment containing the cDNA. The introduced restriction sites, EcoRI at the 5' end and XbaI at the 3' end, are utilised in the following cloning procedures. The gene synthesis fragment is cloned via EcoRI and XbaI into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

8.16 Flow cytometric analysis of cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human Endosialin

In order to test the cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human Endosialin the capability of the constructs to bind to macaque Endosialin and macaque CD3, respectively, is investigated by FACS analysis. For this purpose the macaque Endosialin transfected CHO cells as described in example 8.15 and the macaque T cell line 4119LnPx (kindly provided by Prof Fickenscher, Hygiene Institute, Virology, Erlangen-Nuernberg; published in Knappe A, et al., and Fickenscher H., Blood 2000, 95, 3256-61) are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with with 50 µl of cell culture supernatant of transfected cells expressing the cross-species specific bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti

His antibodies are detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is
5 used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

10 **9. Generation of bispecific single chain antibodies directed at membrane-proximal target epitopes of human IGF-1R**

9.1 Generation of CHO cells expressing human IGF-1R

The coding sequence of human IGF-1R as published in GenBank (Accession number NM_000875) is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for
15 eukaryotic expression of the construct followed by the coding sequence of the human IGF-1R protein and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 424 and 425). The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end,
20 are utilized in the following cloning procedures. An internal restriction site is removed by silent mutation of the coding sequence in the gene synthesis fragment (BspEI: nucleotide 18 from A to C). The gene synthesis fragment is cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The
25 aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR
30 deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

9.2 Generation of a soluble human IGF-1R fusion protein

The modified coding sequence of human IGF-1R as described in Example 9.1 is used for the construction of an artificial cDNA sequence encoding a soluble fusion protein of human IGF-1R and murine IgG1 Fc. To generate a construct for expression of the soluble human IGF-1R fusion protein a cDNA fragment is obtained by gene synthesis according to standard protocols (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 426 and 427). The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the human IGF-1R protein from amino acid 1 to 935 corresponding to the signal peptide and extracellular domains of human IGF-1R, followed in frame by the coding sequence of an artificial Ser₁-Gly₄-Ser₁-linker, followed in frame by the coding sequence of the hinge region and Fc gamma portion of murine IgG1, followed in frame by the coding sequence of a 6 histidine tag and a stop codon. The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are all carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. After two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium (with 4.0 mM L-Glutamine with 0.1% Pluronic F – 68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively a clone of the expression plasmid with sequence-verified nucleotide sequence is used for transfection and protein expression in the FreeStyle 293 Expression System (Invitrogen GmbH, Karlsruhe, Germany) according

to the manufacturer's protocol. Supernatant containing the expressed protein is obtained, cells are removed by centrifugation and the supernatant is stored at -20°C. For purification of the soluble human IGF-1R fusion protein a goat anti-mouse Fc affinity column is prepared according to standard protocols using a commercially available affinity purified goat anti-mouse IgG Fc fragment specific antibody with minimal cross-reaction to human, bovine and horse serum proteins (Jackson ImmunoResearch Europe Ltd.). Using this affinity column the fusion protein is isolated out of cell culture supernatant on an Äkta Explorer System (GE Amersham) and eluted by citric acid. The eluate is neutralized and concentrated.

9.3 Generation of CHO cells expressing murine IGF-1R

The sequence of murine IGF-1R (NM_010513 Mus musculus insulin-like growth factor I receptor (Igf1r), mRNA, National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/entrez>) is used to obtain a synthetic cDNA molecule by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the complete coding sequence of mature murine IGF-1R (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 428 and 429). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

9.4 Generation of CHO cells expressing a mutated human IGF-1R antigen with murine membrane-distal epitopes

The coding sequence of a mutated human IGF-1R antigen with murine membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the coding sequence of the L1 domain and the cysteine-rich domain of mature murine IGF-1R followed in frame by human IGF-1R from the L2 domain to the stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 430 and 431). The gene synthesis fragment is also designed as to introduce restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

9.5 Generation of CHO cells expressing a mutated murine IGF-1R antigen with human membrane-distal epitopes

The coding sequence of a mutated murine IGF-1R antigen with human membrane-distal epitopes is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain the coding sequence of an immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence, followed in frame by the coding sequence of a FLAG tag, followed in frame by the L1 domain and the cysteine-rich domain of mature human IGF-1R followed in frame by murine IGF-1R from the L2 domain to the stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 432 and 433). The gene synthesis fragment is also designed as to introduce

restriction sites at the 5' end (EcoRI) and at the 3' end (Sal I) of the cDNA fragment for cloning into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification for increased antigen expression is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

9.6 Immunization of mice using a soluble human IGF-1R fusion protein

Twelve weeks old F1 mice from BALB/c x C57BL/6 crossings are immunized with the soluble human IGF-1R fusion protein as described in Example 9.2. To this end for each animal 40 µg of the soluble human IGF-1R fusion protein are mixed with 10 nmol of a thioate-modified CpG-Oligonucleotide (5'-tccatgacgttctctgatgct-3') in 300 µl PBS and are injected intraperitoneally. Mice receive booster immunizations after 21, 42 and optionally 63 days in the same way. Ten days after the first booster immunization, blood samples are taken and antibody serum titers against human IGF-1R are tested by flow cytometry according to standard protocols. To this end 200.000 cells of the human IGF-1R transfected CHO cells as described in Example 9.1 are incubated for 30 min on ice with 50 µl of serum of the immunized animals diluted 1:1000 in PBS with 2% FCS. The cells are washed twice in PBS with 2% FCS and binding of serum antibodies is detected with an mouse Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Serum of the animals obtained prior to immunization is used as a negative control. Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002). Animals demonstrating significant serum reactivity against human IGF-1R as determined by the FACS analysis are used in the subsequent experiment.

9.7 Generation of an immune murine antibody scFv library: Construction of a combinatorial antibody library and phage display

Three days after the last booster immunization spleen cells of reactive animals are harvested for the preparation of total RNA according to standard protocols.

- 5 A library of murine immunoglobulin (Ig) light chain (kappa) variable region (VK) and Ig heavy chain variable region (VH) DNA-fragments is constructed by RT-PCR on murine spleen RNA using VK-and VH specific primers. cDNA is synthesized according to standard protocols, see example 2.7.

- 450 ng of the kappa light chain fragments (SacI-SpeI digested) are ligated with 1400
10 ng of the phagemid pComb3H5BHis (SacI-SpeI digested; large fragment). The resulting combinatorial antibody library is then transformed into 300 µl of electrocompetent Escherichia coli XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm, Biorad gene-pulser) resulting in a library size of more than 10^7 independent clones. After one hour of phenotype expression, positive
15 transformants are selected for carbenicillin resistance encoded by the pComb3H5BHis vector in 100 ml of liquid super broth (SB)-culture over night. Cells are then harvested by centrifugation and plasmid preparation is carried out using a commercially available plasmid preparation kit (Qiagen).

- 2800 ng of this plasmid-DNA containing the VK-library (XhoI-BstEII digested; large
20 fragment) are ligated with 900 ng of the heavy chain V-fragments (XhoI-BstEII digested) and again transformed into two 300 µl aliquots of electrocompetent E. coli XL1 Blue cells by electroporation (2.5 kV, 0.2 cm gap cuvette, 25 µFD, 200 Ohm) resulting in a total VH-VK scFv (single chain variable fragment) library size of more than 10^7 independent clones.

- 25 After phenotype expression and slow adaptation to carbenicillin, the E. coli cells containing the antibody library are transferred into SB-Carbenicillin (SB with 50 µg/mL carbenicillin) selection medium. The E. coli cells containing the antibody library are then infected with an infectious dose of 10^{12} particles of helper phage VCSM13 resulting in the production and secretion of filamentous M13 phage,
30 wherein each phage particle contains single stranded pComb3H5BHis-DNA encoding a murine scFv-fragment and displays the corresponding scFv-protein as a translational fusion to phage coat protein III. This pool of phages displaying the antibody library is later used for the selection of antigen binding entities.

9.8 Phage display based selection of membrane-proximal target binders on CHO cells expressing the mutated human IGF-1R antigen with murine membrane-distal epitopes

The phage library carrying the cloned scFv-repertoire is harvested from the
5 respective culture supernatant by PEG8000/NaCl precipitation and centrifugation.
Approximately 10^{11} to 10^{12} scFv phage particles are resuspended in 0.4 ml of
PBS/0.1% BSA and incubated with 10^5 to 10^7 CHO cells expressing the mutated
human IGF-1R antigen with murine membrane-distal epitopes as described in
example 9.4 for 1 hour on ice under slow agitation. These CHO cells are grown
10 beforehand, harvested by centrifugation, washed in PBS and resuspended in PBS/1
% FCS (containing Na Azide). scFv phage which do not specifically bind to the CHO
cells are eliminated by up to five washing steps with PBS/1 % FCS (containing Na
Azide). After washing, binding entities are eluted from the cells by resuspending the
cells in HCl-glycine pH 2.2 (10 min incubation with subsequent vortexing) and after
15 neutralization with 2 M Tris pH 12, the eluate is used for infection of a fresh
uninfected E. coli XL1 Blue culture ($OD_{600} > 0.5$). The E. coli culture containing E.
coli cells successfully transduced with a phagemid copy, encoding a murine scFv-
fragment, are again selected for carbenicillin resistance and subsequently infected
with VCMS 13 helper phage to start the second round of antibody display and in vitro
20 selection. Typically a total of 4 to 5 rounds of selections are carried out.

9.9 Screening for membrane-proximal target binders on CHO cells expressing the human IGF-1R antigen, the murine IGF-1R antigen and the mutated murine IGF-1R antigen with human membrane-distal epitopes

Plasmid DNA corresponding to 4 and 5 rounds of panning is isolated from E. coli
25 cultures after selection. For the production of soluble scFv-protein, VH-VL-DNA
fragments are excised from the plasmids (XhoI-SpeI). These fragments are cloned
via the same restriction sites in the plasmid pComb3H5BFlag/His differing from the
original pComb3H5BHis in that the expression construct (e.g. scFv) includes a Flag-
tag (TG DYK D D D D K) between the scFv and the His6-tag and the additional phage
30 proteins are deleted. After ligation, each pool (different rounds of panning) of plasmid
DNA is transformed into 100 μ l heat shock competent E. coli TG1 or XLI blue and
plated onto carbenicillin LB-agar. Single colonies are picked into 100 μ l of LB carb
(LB with 50 μ g/ml carbenicillin).

After induction with 1 mM IPTG E. coli transformed with pComb3H5BFlag/His containing a VL-and VH-segment produce soluble scFv in sufficient amounts. Due to a suitable signal sequence, the scFv is exported into the periplasma where it folds into a functional conformation.

- 5 Single E. coli bacterial colonies from the transformation plates are picked for periplasmic small scale preparations and grown in SB-medium (e.g. 10 ml) supplemented with 20 mM MgCl₂ and carbenicillin 50µg/ml (and re-dissolved in PBS (e.g. 1 ml) after harvesting. A temperature shock is applied by four rounds of freezing at -70°C and thawing at 37°C whereby the outer membrane of the bacteria is
10 destroyed and the soluble periplasmic proteins including the scFvs are released into the supernatant. After elimination of intact cells and cell-debris by centrifugation, the supernatant containing the murine anti-human IGF-1R-scFvs is collected and used for further examination.

- Screening of the isolated scFvs for membrane-proximal target binders is performed
15 by flow cytometry on CHO cells expressing the human IGF-1R antigen as described in Example 9.1, the murine IGF-1R antigen as described in Example 9.3 and the mutated murine IGF-1R antigen with human membrane-distal epitopes as described in Example 9.5.

- For flow cytometry 2.5×10^5 cells of the respective cell lines are incubated with 50 µl
20 supernatant. The binding of the constructs is detected with an anti-His antibody (Penta-His Antibody, BSA free, Qiagen GmbH, Hilden, FRG) at 2 µg/ml in 50 µl PBS with 2% FCS. As a second step reagent a R-Phycoerythrin-conjugated affinity purified F(ab')₂ fragment, goat anti-mouse IgG (Fc-gamma fragment specific), diluted 1:100 in 50 µl PBS with 2% FCS (Dianova, Hamburg, FRG) is used. The samples are
25 measured on a FACSScan (BD biosciences, Heidelberg, FRG).

Only constructs which show binding to CHO cells expressing the human IGF-1R antigen and do not show binding to CHO cells expressing the murine IGF-1R antigen and also do not show binding to CHO cells expressing the mutated murine IGF-1R antigen with human membrane-distal epitopes are selected for further use.

30 **9.10 Generation of human/humanized equivalents of non-human scFvs to membrane-proximal target epitopes of human IGF-1R**

The VH region of a murine anti-IGF-1R scFv to a membrane-proximal target epitope of human IGF-1R is aligned against human antibody germline amino acid sequences.

The human antibody germline VH sequence is chosen which has the closest homology to the non-human VH and a direct alignment of the two amino acid sequences is performed. There are a number of framework residues of the non-human VH that differ from the human VH framework regions ("different framework positions"). Some of these residues may contribute to the binding and activity of the antibody to its target.

To construct a library that contains the murine CDRs and at every framework position that differs from the chosen human VH sequence both possible residues (the human and the maternal murine amino acid residue), degenerated oligonucleotides are synthesized. These oligonucleotides incorporate at the differing positions the human residue with a probability of 75 % and the murine residue with a probability of 25 %. For one human VH e.g. six of these oligonucleotides have to be synthesized that overlap in a terminal stretch of approximately 20 nucleotides. To this end every second primer is an antisense primer. Restriction sites within the oligonucleotides needed for later cloning are deleted.

These primers may have a length of 60 to 90 nucleotides, depending on the number of primers that are needed to span over the whole V sequence.

These e.g. six primers are mixed in equal amounts (e.g. 1 µl of each primer (primer stocks 20 to 100 µM) to a 20 µl PCR reaction) and added to a PCR mix consisting of PCR buffer, nucleotides and Taq polymerase. This mix is incubated at 94 °C for 3 minutes, 65 °C for 1 minute, 62°C for 1 minute, 59 °C for 1 minute, 56 °C for 1 minute, 52 °C for 1 minute, 50 °C for 1 minute and at 72°C for 10 minutes in a PCR cycler. Subsequently the product is run in an agarose gel electrophoresis and the product of a size from 200 to 400 base pairs isolated from the gel according to standard methods.

This PCR product is then used as a template for a standard PCR reaction using primers that incorporate suitable N-terminal and C-terminal cloning restriction sites. The DNA fragment of the correct size (for a VH approximately 350 nucleotides) is isolated by agarose gel electrophoresis according to standard methods. In this way sufficient VH DNA fragment is amplified. This VH fragment is now a pool of VH fragments that have each one a different amount of human and murine residues at the respective differing framework positions (pool of humanized VH). The same procedure is performed for the VL region of the murine anti-IGF-1R scFv to a membrane-proximal target epitope of human IGF-1R (pool of humanized VL).

The pool of humanized VH is then combined with the pool of humanized VL in the phage display vector pComb3H5Bhis to form a library of functional scFvs from which – after display on filamentous phage - anti-IGF-1R binders to membrane-proximal target epitopes of human IGF-1R are selected, screened, identified and confirmed as described above for the parental non-human (murine) anti-IGF-1R scFv. Single clones are then analyzed for favorable properties and amino acid sequence. Those scFvs, which are closest in amino acid sequence homology to human germline V-segments, are preferred.

Human/humanized anti-IGF-1R scFvs to membrane-proximal target epitopes of human IGF-1R are converted into recombinant bispecific single chain antibodies and further characterized as follows.

9.11 Generation of I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human IGF-1R

Anti-IGF-1R scFvs to membrane-proximal target epitopes of human IGF-1R with favorable properties and amino acid sequence are converted into recombinant bispecific single chain antibodies by joining them via a Gly₄Ser₁-linker with the CD3 specific scFv I2C (SEQ ID) to result in constructs with the domain arrangement VH_{IGF-1R} - (Gly₄Ser₁)₃ - VL_{IGF-1R} - Ser₁Gly₄Ser₁ - VH_{CD3} - (Gly₄Ser₁)₃ - VL_{CD3}.

I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human IGF-1R were designed as set out in the following Table 8:

Table 8: Formats of I2C-based bispecific single chain antibodies directed at membrane-proximal target epitopes of human IGF-1R

SEQ ID (nucl/prot)	Formats of protein constructs (N → C)
848/847	IGF1R12HLxI2CHL
862/861	IGF1R24HLxI2CHL

Alternatively further constructs with different domain arrangements can be generated according to standard protocols. For expression in CHO cells the coding sequences of (i) an N-terminal immunoglobulin heavy chain leader comprising a start codon embedded within a Kozak consensus sequence and (ii) a C-terminal His₆-tag followed by a stop codon are both attached in frame to the nucleotide sequence

encoding the bispecific single chain antibodies prior to insertion of the resulting DNA-fragment as obtained by gene synthesis into the multiple cloning site of the expression vector pEF-DHFR (Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). A clone with sequence-verified nucleotide sequence is transfected into
5 DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

10 **9.12 Expression and purification of bispecific single chain antibody molecules directed at membrane-proximal target epitopes of human IGF-1R**

Bispecific single chain antibody molecules are expressed in Chinese hamster ovary cells (CHO). Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene
15 amplification of the constructs is induced by addition of increasing concentrations of MTX up to final concentrations of 20 nM MTX. After two passages of stationary culture cell culture supernatant is collected and used in the subsequent experiments. To generate supernatant for purification after two passages of stationary culture the cells are grown in roller bottles with nucleoside-free HyQ PF CHO liquid soy medium
20 (with 4.0 mM L-Glutamine with 0.1% Pluronic F-68; HyClone) for 7 days before harvest. The cells are removed by centrifugation and the supernatant containing the expressed protein is stored at -20°C. Alternatively, constructs are transiently expressed in HEK 293 cells. Transfection is performed with 293fectin reagent (Invitrogen, #12347-019) according to the manufacturer's protocol. Furthermore the
25 constructs are alternatively expressed in transiently transfected DHFR deficient CHO cells using for example FuGENE® HD Transfection Reagent (Roche Diagnostics GmbH, Cat. No. 04709691001) according to the manufacturer's protocol.

Äkta® Explorer System (GE Health Systems) and Unicorn® Software are used for chromatography. Immobilized metal affinity chromatography ("IMAC") is performed
30 using a Fractogel EMD chelate® (Merck) which is loaded with ZnCl₂ according to the protocol provided by the manufacturer. The column is equilibrated with buffer A (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl) and the cell culture supernatant (500 ml) is applied to the column (10 ml) at a flow rate of 3 ml/min. The column is washed with buffer A to remove unbound sample. Bound protein is eluted using a

two step gradient of buffer B (20 mM sodium phosphate buffer pH 7.2, 0.1 M NaCl, 0.5 M Imidazole) according to the following procedure:

Step 1: 20% buffer B in 6 column volumes

Step 2: 100% buffer B in 6 column volumes

- 5 Eluted protein fractions from step 2 are pooled for further purification. All chemicals are of research grade and purchased from Sigma (Deisenhofen) or Merck (Darmstadt).

Gel filtration chromatography is performed on a HiLoad 16/60 Superdex 200 prep grade column (GE/Amersham) equilibrated with Equi-buffer (25 mM Citrate, 200 mM
10 Lysine, 5% Glycerol, pH 7.2). Eluted protein samples (flow rate 1 ml/min) are subjected to standard SDS-PAGE and Western Blot for detection. Prior to purification, the column is calibrated for molecular weight determination (molecular weight marker kit, Sigma MW GF-200). Protein concentrations are determined using OD280 nm.

- 15 Purified bispecific single chain antibody protein is analyzed in SDS PAGE under reducing conditions performed with pre-cast 4-12% Bis Tris gels (Invitrogen). Sample preparation and application are performed according to the protocol provided by the manufacturer. The molecular weight is determined with MultiMark protein standard (Invitrogen). The gel is stained with colloidal Coomassie (Invitrogen protocol). The
20 purity of the isolated protein is typically >95% as determined by SDS-PAGE.

The bispecific single chain antibody has a molecular weight of about 52 kDa under native conditions as determined by gel filtration in PBS.

- Western Blot is performed using an Optitran® BA-S83 membrane and the Invitrogen Blot Module according to the protocol provided by the manufacturer. The antibody
25 used is directed against the His Tag (Penta His, Qiagen) and a Goat-anti-mouse Ig labeled with alkaline phosphatase (AP) (Sigma) is used as second step reagent, and BCIP/NBT (Sigma) as substrate. A band detected at 52 kD corresponds to purified bispecific single chain antibodies.

30 **9.13 Flow cytometric binding analysis of bispecific antibodies directed at membrane-proximal target epitopes of human IGF-1R**

In order to test the functionality of bispecific antibody constructs regarding the capability to bind to CD3 and to membrane-proximal target epitopes of human IGF-1R, respectively, a FACS analysis is performed. For this purpose CHO cells transfected with human IGF-1R as described in Example 9.1 and the human CD3

positive T cell leukemia cell line HPB-ALL (DSMZ, Braunschweig, ACC483) are used. For confirmation of binding to membrane-proximal target epitopes of human IGF-1R - in addition - CHO cells expressing the murine IGF-1R antigen as described in Example 9.3, CHO cells expressing the mutated human IGF-1R antigen with murine membrane-distal epitopes as described in Example 9.4 and CHO cells expressing the mutated murine IGF-1R antigen with human membrane-distal epitopes as described in Example 9.5 are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with 50 µl of cell culture supernatant of transfected cells expressing the bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound anti His antibodies are detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

Only those constructs that show bispecific binding to human CD3 as well as to human IGF-1R and neither bind to the murine IGF-1R antigen nor to the mutated murine IGF-1R antigen with human membrane-distal epitopes are selected for further use.

The bispecific binding of the single chain molecules listed above was clearly detectable as shown in Figure 17. In the FACS analysis all constructs showed binding to human CD3 and human IGF-1R compared to the negative control.

Membrane-proximal target binding of the single chain molecules listed above was clearly detectable as shown in Figure 19. In the FACS analysis all constructs showed binding to the mutated human IGF-1R antigen with murine membrane-distal epitopes and did not show binding to the murine IGF-1R antigen and did also not show binding to the mutated murine IGF-1R antigen with human membrane-distal epitopes as compared to the negative control.

9.14 Bioactivity of bispecific antibodies directed at membrane-proximal target epitopes of human IGF-1R

Bioactivity of generated bispecific single chain antibodies is analyzed by chromium 51 (⁵¹Cr) release in vitro cytotoxicity assays using the CHO cells transfected with human IGF-1R described in Example 9.1. As effector cells stimulated human CD4/CD56 depleted PBMC are used.

Stimulated human PBMC are obtained as follows:

A Petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) is coated with a commercially available anti-CD3 specific antibody (e.g. OKT3, Orthoclone) in a final concentration of 1 µg/ml for 1 hour at 37°C. Unbound protein is removed by one washing step with PBS. The fresh PBMC are isolated from peripheral blood (30 – 50 ml human blood) by Ficoll gradient centrifugation according to standard protocols. 3 - 5 x 10⁷ PBMC are added to the precoated petri dish in 120 ml of RPMI 1640 with stabilized glutamine / 10% FCS / IL-2 20 U/ml (Proleukin, Chiron) and stimulated for 2 days. On the third day the cells are collected and washed once with RPMI 1640. IL 2 is added to a final concentration of 20 U/ml and the cells are cultivated again for one day in the same cell culture medium as above.

By depletion of CD4+ T cells and CD56+ NK cells according to standard protocols CD8+ cytotoxic T lymphocytes (CTLs) are enriched.

Target cells are washed twice with PBS and labeled with 11.1 MBq ⁵¹Cr in a final volume of 100µl RPMI with 50% FCS for 60 minutes at 37°C. Subsequently the labeled target cells are washed 3 times with 5 ml RPMI and then used in the cytotoxicity assay. The assay is performed in a 96 well plate in a total volume of 250µl supplemented RPMI (as above) with an E:T ratio of 10:1. Supernatant of cells expressing the bispecific single chain antibody molecules in a final concentration of 50% and 20 twofold dilutions thereof are applied. The assay time is 18 hours. Cytotoxicity is measured as relative values of released chromium in the supernatant related to the difference of maximum lysis (addition of Triton-X) and spontaneous lysis (without effector cells). All measurements are done in quadruplicates. Measurement of chromium activity in the supernatants is performed with a Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany). Analysis of the experimental data is performed with Prism 4 for Windows (version 4.02, GraphPad Software Inc., San Diego, California, USA). Sigmoidal dose response curves typically have R² values >0.90 as determined by the software.

Only those constructs showing potent recruitment of cytotoxic activity of effector T cells against target cells positive for IGF-1R are selected for further use.

9.15 Generation of CHO cells expressing macaque IGF-1R

The coding sequence of macaque IGF-1R as published in GenBank (Accession number XM_001100407) is obtained by gene synthesis according to standard protocols. The gene synthesis fragment is designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the macaque IGF-1R protein and a stop codon (the cDNA and amino acid sequence of the construct is listed under SEQ ID Nos 434 and 435). The gene synthesis fragment is also designed as to introduce restriction sites at the beginning and at the end of the fragment. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end, are utilized in the following cloning procedures. The gene synthesis fragment is cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150) following standard protocols. The aforementioned procedures are carried out according to standard protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence is transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells is performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct is induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

9.16 Flow cytometric analysis of cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human IGF-1R

In order to test the cross-species specificity of bispecific antibodies directed at membrane-proximal target epitopes of human IGF-1R the capability of the constructs to bind to macaque IGF-1R and macaque CD3, respectively, is investigated by FACS analysis. For this purpose the macaque IGF-1R transfected CHO cells as described in example 9.15 and the macaque T cell line 4119LnPx (kindly provided by Prof Fickenscher, Hygiene Institute, Virology, Erlangen-Nuernberg; published in Knappe A, et al., and Fickenscher H., Blood 2000, 95, 3256-61) are used. 200.000 cells of the respective cell lines are incubated for 30 min on ice with with 50 µl of cell culture

supernatant of transfected cells expressing the cross-species specific bispecific antibody constructs. The cells are washed twice in PBS with 2% FCS and binding of the construct is detected with a murine Penta His antibody (Qiagen; diluted 1:20 in 50 μ l PBS with 2% FCS). After washing, bound anti His antibodies are detected with an

5 Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS. Supernatant of untransfected cells is used as a negative control.

Flow cytometry is performed on a FACS-Calibur apparatus, the CellQuest software is used to acquire and analyze the data (Becton Dickinson biosciences, Heidelberg). FACS staining and measuring of the fluorescence intensity are performed as

10 described in Current Protocols in Immunology (Coligan, Kruisbeek, Margulies, Shevach and Strober, Wiley-Interscience, 2002).

The cross-species specific binding of the single chain molecules listed above was clearly detectable as shown in Figure 17. In the FACS analysis all constructs showed binding to macaque CD3 and macaque IGF-1R compared to the negative control.

15 Human/humanized equivalents of scFvs specific for IGF-1R contained in the bispecific single chain molecules are generated as described herein. Cloning of binding molecules based on these human/humanized scFvs and expression and purification of these bispecific single chain molecules is performed as described above. Flow cytometric analysis of bispecific binding and analysis of bioactivity by

20 chromium 51 (^{51}Cr) release in vitro cytotoxicity assays is performed as described above. Based on demonstrated bispecific binding and recruited cytotoxicity binding molecules are selected for further use.

Example 10

10.1. Generation of PSMA- and CD3-directed bispecific single chain antibodies

25 Bispecific single chain antibodies comprising either scFv binding domain P7 against a PSMA-epitope with $< 60\text{\AA}$ membrane-distance or scFv binding domain D4 against a PSMA-epitope with $\geq 60\text{\AA}$ membrane-distance and the scFv binding domain I2C directed at CD3epsilon on human T cells were obtained by gene synthesis. The gene synthesis fragments were designed as to contain first a Kozak site for eukaryotic

30 expression of the construct, followed by a 19 amino acid immunoglobulin leader peptide, followed in frame by the coding sequence of the bispecific single chain antibody, followed in frame by the coding sequence of a 6 histidine tag and a stop

codon. The variable region arrangements as well as the SEQ ID Nos of the cDNA- and amino acid sequences are listed in the table 9 below.

SEQ ID (nucl/prot)	Formats of protein constructs (N → C)
488/487	PSMA-P7 HL x I2C HL
474/473	PSMA-D4 HL x I2C HL

- 5 The gene synthesis fragments were also designed as to introduce suitable restriction sites at the beginning (EcoRI) and at the end of the fragment (Sal I) for cloning of the gene synthesis fragment into the mammalian cell expression vector pEF-DHFR (pEF-DHFR is described in Raum et al. Cancer Immunol Immunother 50 (2001) 141-150). The aforementioned procedures were carried out according to standard
- 10 protocols (Sambrook, Molecular Cloning; A Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence was transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufmann R.J. (1990)
- 15 Methods Enzymol. 185, 537-566. Gene amplification of the construct was induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX. After two passages of stationary culture cell culture supernatant was collected and used in the subsequent experiments.

20 **10.2 Epitope mapping of the PSMA- and CD3-reactive bispecific single chain antibody molecule PSMA-P7 HL x I2C HL**

A PSMA-epitope with < 60Å membrane-distance of bispecific single chain antibody PSMA-P7 HL x I2C HL was confirmed by epitope mapping using chimeric PSMA constructs.

10.2.1 Generation of CHO cells expressing human / rat PSMA chimeras

- 25 PSMA of rattus norvegicus, which is not bound by PSMA bispecific single chain antibody PSMA-P7 HL x I2C HL, was used for making chimeras with human PSMA. Thus, creating a chimera in the region containing the binding epitope of a PSMA bispecific single chain antibody leads to loss of binding of said bispecific single chain antibody to the respective PSMA construct.

The coding sequence of human PSMA as published in GenBank (Accession number NM_004476) and the coding sequence of rat PSMA (NM_057185, *Rattus norvegicus* folate hydrolase (Folh1), mRNA, National Center for Biotechnology Information, <http://www.ncbi.nlm.nih.gov/entrez>) were used for generation of the chimeric constructs.

A set of 6 chimeric cDNA constructs was designed and generated by gene synthesis according to standard protocols. In the constructs segments of the coding sequences for the amino acids 140 to 169, 281 to 284, 300 to 344, 589 to 617, 683 to 690 and 716 to 750, respectively, were exchanged for the homologous sequences of rat PSMA.

Chimeric PSMA constructs were generated as described above and designated as set out in the following Table 10:

Table 10: Designation of chimeric PSMA constructs

SEQ ID (nucl/prot)	Designation
461/462	huPSMArat140-169
463/464	huPSMArat281-284
465/466	huPSMArat300-344
467/468	huPSMArat598-617
469/470	huPSMArat683-690
471/472	huPSMArat716-750

The gene synthesis fragments were designed as to contain first a Kozak site for eukaryotic expression of the construct followed by the coding sequence of the chimeric PSMA proteins, followed in frame by the coding sequence of a FLAG-tag and a stop codon. The gene synthesis fragments were also designed as to introduce restriction sites at the beginning and at the end of the fragments. The introduced restriction sites, EcoRI at the 5' end and Sall at the 3' end, were utilized in the following cloning procedures. Undesirable internal restriction sites were removed by silent mutation of the coding sequence in the gene synthesis fragments. The gene synthesis fragments were cloned via EcoRI and Sall into a plasmid designated pEF-DHFR (pEF-DHFR is described in Raum et al. *Cancer Immunol Immunother* 50 (2001) 141-150) following standard protocols. The aforementioned procedures were carried out according to standard protocols (Sambrook, *Molecular Cloning*; A

Laboratory Manual, 3rd edition, Cold Spring Harbour Laboratory Press, Cold Spring Harbour, New York (2001)). A clone with sequence-verified nucleotide sequence was transfected into DHFR deficient CHO cells for eukaryotic expression of the construct. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufmann R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the construct was induced by increasing concentrations of methotrexate (MTX) to a final concentration of up to 20 nM MTX.

10.2.2 Flow cytometric binding analysis for epitope mapping of the PSMA- and CD3-reactive bispecific single chain antibody molecule PSMA-P7 HL x I2C HL using chimeric PSMA proteins

In order to determine the binding epitope of the PSMA bispecific single chain antibody PSMA-P7 HL x I2C HL a FACS analysis was performed. For this purpose CHO cells transfected with human / rat chimeric PSMA molecules as described in Example 10.2.1 above were used. FACS analysis with supernatant of CHO cells expressing PSMA-P7 HL x I2C HL was performed as described herein. Detection of binding of PSMA-P7 HL x I2C HL was performed using a murine Penta His antibody and as second step reagent an Fc gamma-specific antibody conjugated to phycoerythrin. Supernatant of untransfected cells was used as a negative control. Supernatant of CHO cells expressing the bispecific single chain antibody construct PSMA-D4 HL x I2C HL cross-reactive with rat PSMA was used as control for expression of the chimeric PSMA constructs.

As shown in Figure 20 both PSMA bispecific single chain antibodies, PSMA-P7 HL x I2C HL and PSMA-D4 HL x I2C HL, showed binding to the chimeric constructs huPSMArat140-169, huPSMArat281-284, huPSMArat300-344, huPSMArat683-690 and huPSMArat716-750. As furthermore shown in Figure 20 there is a lack of binding for PSMA-P7 HL x I2C HL to the construct huPSMArat598-617, which demonstrates the presence of its binding epitope in the region of amino acids 598 to 617 of human PSMA.

As shown in Table 1 the amino acids 598 to 617 constitute a membrane proximal epitope as defined herein. In conclusion the results of the mapping based on chimeric PSMA constructs demonstrate that bispecific single-chain antibody PSMA-P7 HL x I2C HL recognizes a membrane-proximal target epitope of human PSMA with < 60Å membrane-distance as defined herein.

10.3 Epitope mapping of the PSMA- and CD3-reactive bispecific single chain antibody molecule PSMA-D4 HL x I2C HL

A PSMA-epitope with $\geq 60\text{\AA}$ membrane-distance of bispecific single chain antibody PSMA-D4 HL x I2C HL was confirmed by epitope mapping using a peptide scanning approach. Peptide scanning uses overlapping peptides of a given protein and analyses antibody binding to immobilized peptides by enzyme-linked immunosorbent assays (ELISAs). The epitope mapping experiments with the PSMA bispecific single chain antibody PSMA-D4 HL x I2C HL were performed as described in detail in Bernard et al. 2004, J. Biol. Chem., 279: 24313 -22 and Teeling et al. 2006, J Immunol., 177: 362-71.

In brief, 693 different 15-mer peptides were synthesized that span the entire extracellular amino acid sequence of human PSMA and overlap with each neighboring 15-mer peptide by 14 amino acids. These peptides were coated to ELISA wells in a 384-well plate format. For this series of experiments, the anti-PSMA scFv fragment of bispecific single chain antibody PSMA-D4 HL x I2C HL was produced in E. coli and used for ELISA as crude periplasmic extracts prepared as described herein. The scFv antibody was incubated with the peptides and specific binding detected using an anti-His antibody. Binding signals were measured in a 384-well ELISA reader. As shown in the Figure 21 a clear maximum signal was obtained for a peptide spanning over the amino acids threonine 334 to threonine 339, which demonstrates the presence of binding epitope of PSMA-D4 HL x I2C HL in the region of amino acids 334 to 339 of human PSMA. As shown in Table 1 threonine 334 and threonine 339 constitute a membrane-distal epitope as defined herein.

In conclusion the results of the mapping based on peptide scanning demonstrate that bispecific single-chain antibody PSMA-D4 HL x I2C HL recognizes a membrane-distal target epitope of human PSMA with $\geq 60\text{\AA}$ membrane-distance as defined herein.

10.4 Comparative analysis of cytotoxic activity of bispecific antibodies single-chain antibodies directed at membrane-proximal and membrane-distal target epitopes of human PSMA

Bioactivity of bispecific single chain antibodies was analyzed by a CytoTox-GloTM cytotoxicity assay with unstimulated human PBMC using the CHO cells transfected with human PSMA described in Example 2.1. As effector cells unstimulated human PBMC were used.

Unstimulated human PBMC were obtained as follows:

Human peripheral blood mononuclear cells (PBMC) were prepared by Ficoll density gradient centrifugation from enriched lymphocyte preparations (buffy coats), a side product of blood banks collecting blood for transfusions. Buffy coats were supplied by a local blood bank and PBMC were prepared on the same day of blood collection. After Ficoll density centrifugation and extensive washes with Dulbecco's PBS (Gibco), remaining erythrocytes were removed from PBMC via incubation with erythrocyte lysis buffer (155 mM NH₄Cl, 10 mM KHCO₃, 100 μM EDTA). Platelets were removed via the supernatant upon centrifugation of PBMC at 100xg. Remaining lymphocytes mainly encompass B and T lymphocytes, NK cells and monocytes. PBMC were kept in culture at 37°C and 5% CO₂ in RPMI medium (Gibco) with 10% FBS (Gibco). All procedures were performed according to standard protocols (Current Protocols in Immunology; Coligan, Kruisbeek, Margulies, Shevach and Strober; Wiley-Interscience, 2002)

The CytoTox-Glo™ cytotoxicity assay (Kit from Promega) was used according to the instructions provided by the manufacturer.

Each measurement was performed in triplicates with defined dilution series of purified PSMA specific bispecific antibodies (0.001 ng/ml to 250 ng/ml) and appropriate controls to define spontaneous lysis (effector and target cells without bispecific antibodies) and maximum lysis (addition of detergent digitonin to cells). 10000/well target and 100000/well effector cells were mixed in a defined ratio with effector cells in excess (E:T ratio of 10:1). After incubation at 37°C for 20-24 hours CytoTox-Glo cytotoxicity assay reagent (AAF-Glo™; part of the CytoTox-Glo Kit from Promega) was added to all wells. The cells and the reagent were mixed by orbital shaking and incubated at room temperature for 1 hour. Determination of the number of dead cells was performed subsequently by measuring luminescence with a plate reader (TECAN Spectrafluorometer). The measured signal correlates directly with the amount of lysed cells.

On the basis of those measured values the cytotoxicity values of every individual sample were calculated according to following formula:

$$S [\%] = (V - B) / (M - B)$$

Wherein S is specific toxicity, V is the measured value, B is the average of blank values and M is the average of maximum lysis.

As shown in Figure 22 the bispecific antibody PSMA-P7 HL x I2C HL directed at a membrane-proximal target epitope of human PSMA (as shown in Example 10.2)
5 demonstrated superior cytotoxic activity against human PSMA positive target cells as compared to the bispecific single-chain antibody PSMA-D4 HL x I2C HL directed at membrane-distal target epitope of human PSMA (as shown in Example 10.3).

Table 1:

aa pos.	HUM	RAT	distance to aa of reference (Å)
63	D	Q	11,7
79	Q	R	35,75
80	I	T	37,16
87	E	Q	40,16
88	Q	H	37,46
91	Q	E	33,93
97	Q	H	25,2
98	S	A	24,96
107	S	L	21,07
111	A	S	31,1
112	H	D	34,65
140	N	K	62,43
144	F	A	51,27
146	P	L	50,14
147	P	S	47,22
154	V	I	44,73
157	I	V	44,61
161	F	Y	48,85
169	M	T	62,96
191	D	V	68,07
207	K	V	46,97
225	V	I	56,52
236	A	V	45,34
258	I	V	46,85
281	R	H	50,31
282	G	E	52,65
283	I	F	52,92
284	A	T	55,82

300	Y	D	56,23
308	K	H	67,6
320	R	K	58,61
322	S	G	56,59
334	T	A	73,45
339	T	K	75,1
344	M	L	60,7
349	T	Y	52,3
351	E	K	48,1
363	R	K	15,8
380	S	A	35,47
401	S	T	11,76
408	E	K	7,93
438	N	H	31,19
471	Y	H	25,5
475	H	Y	23,2
482	K	P	20,44
495	E	D	27,54
498	T	K	30,45
499	K	E	33,18
504	P	T	38,15
507	S	T	35,93
542	E	K	42,23
543	T	N	42,97
546	F	V	43,56
548	G	S	39,02
569	M	T	28,15
582	G	A	14,07
598	R	Q	31,22
599	D	S	30,9

603	V	A	36,05
605	R	K	40,9
607	Y	H	41,38
609	D	E	45,95
610	K	T	45,84
613	S	N	51,09
617	K	N	56,75
624	T	A	64,88
626	S	M	60,97
627	V	I	57,43
637	K	N	42,63
641	E	D	38,71
642	I	V	36,92
647	S	N	29,22
648	E	Q	30,75
653	F	L	26,85
660	V	L	26,27
663	M	I	26,27
664	M	L	26,99
670	F	Y	28,76
683	D	G	49,46
690	V	I	40,04
716	E	N	60,95
717	S	N	62,54
721	P	T	64,1
726	G	R	58,11
733	Y	S	49,53
734	V	I	46,73
747	S	R	35,94
750	A	D	38,19

Table 2

aa pos.	HUM	MU	distance to aa of reference (Å)
57	F	Y	30,42
64	G	E	46,67
72	A	E	30,96
74	N	D	35,61
78	L	F	42,46
84	G	R	42,58
85	Q	E	43,72
88	T	I	43,24
93	R	S	43,31
101	S	T	38,96
102	N	D	40,41
136	S	Q	55,96
144	N	Y	61,64
185	F	Y	64,38
186	N	T	63,68
191	K	R	57,71
217	N	D	58,09
224	A	V	56,62
229	T	S	60,5
233	V	I	54,23
242	E	G	49,32
264	I	V	59,95
267	I	V	63,87
273	A	H	70,89
274	Y	H	72,78
278	Q	M	66,9

284	A	E	67,3
300	T	S	59
301	D	S	60,96
328	Q	H	72,16
329	T	A	69,98
331	D	E	68,26
335	T	N	67,63
339	I	V	59,27
356	V	A	50,4
359	Y	Q	56,3
362	I	T	60,49
399	N	Y	41,15
417	E	G	35,94
431	S	N	56,56
432	Y	S	57,15
457	D	Y	47,06
458	Y	K	48,96
471	I	L	19,07
486	K	Q	33,08
487	I	V	29,68
497	A	S	11,55
499	K	R	7,95
506	E	V	18,65
512	E	K	37,64
513	V	D	39,8
514	D	G	43,57
515	E	G	44,32
516	I	L	41,48

518	L	F	34,87
550	R	K	27,19
559	S	T	15,7
567	M	I	19,02
584	L	F	42,67
586	Y	H	46,36
601	I	L	36,05
616	K	E	31,12
659	V	I	49,32
661	T	S	47,68
736	L	I	32,18
739	L	R	28,95
741	T	Q	27,16

SEQ ID NO.	DESIGNATION	SOURCE	TYPE	SEQUENCE
1.	Human CD3 ϵ extracellular domain	human	aa	QDGNNEEMGGITQTTPYKVSISGTTVLTICPQYPGSEILLQHNNDKNI GGDEDDKNI GSDEDDHLSLKE FSELEQSGYVVCYPRGSKPEDANFYLYLRVCENCMEMD
2.	Human CD3 ϵ 1-27	human	aa	QDGNNEEMGGITQTTPYKVSISGTTVILT
3.	<i>Calithrix jacchus</i> CD3 ϵ extracellular domain	<i>Calithrix jacchus</i>	aa	QDGNNEEMGDTTQNPYKVSISGTTVTILTCPRYDGHEIKWLVNSQKKEGHEDHLLLEDFSEMEQSGY YACLSKETPAEEASHYLYLKARVCENCVEVD
4.	<i>Calithrix jacchus</i> CD3 ϵ 1-27	<i>Calithrix jacchus</i>	aa	QDGNNEEMGDTTQNPYKVSISGTTVTILT
5.	<i>Saguinus oedipus</i> CD3 ϵ extracellular domain	<i>Saguinus oedipus</i>	aa	QDGNNEEMGDTTQNPYKVSISGTTVTILTCPRYDGHEIKWLVNSQKKEGHEDHLLLEDFSEMEQSGY YACLSKETPAEEASHYLYLKARVCENCVEVD
6.	<i>Saguinus oedipus</i> CD3 ϵ 1-27	<i>Saguinus oedipus</i>	aa	QDGNNEEMGDTTQNPYKVSISGTTVTILT
7.	<i>Saimiri sciureus</i> CD3 ϵ extracellular domain	<i>Saimiri sciureus</i>	aa	QDGNNEEIGDTPQNPYKVSISGTTVTILTCPRYDGOEIKWLVNDQKKEGHEDHLLLEDFSEMEQSGY YACLSKETPTEEASHYLYLKARVCENCVEVD
8.	<i>Saimiri sciureus</i> CD3 ϵ 1-27	<i>Saimiri sciureus</i>	aa	QDGNNEEIGDTPQNPYKVSISGTTVTILT
9.	CDR-L1 of F6A	artificial	aa	GSSTGAVTSGYY ϵ N
10.	CDR-L2 of F6A	artificial	aa	GTKFLAP
11.	CDR-L3 of F6A	artificial	aa	ALWYSNRWV
12.	CDR-H1 of F6A	artificial	aa	IYAMN
13.	CDR-H2 of F6A	artificial	aa	RIRSKYNNYATYYADSVKS
14.	CDR-H3 of F6A	artificial	aa	HGNFGNSYVSFFAY
15.	VH of F6A	artificial	aa	EVQLVESSGGLVQPPGGSILKLSCAASGFTFNIYAMNWVRQAPGKLEWVARIRSKYNNYATYYADS VKSRFTISRDDSKNTAYLQMNLIKTEDTAVYYCVRHGNFGNSYVSFFAYWGQGTLLVTVSS
16.	VH of F6A	artificial	nt	GAGGTGCAGCTGGTCGAGTCCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAAGCCTCTGGATTCACTTCAATATCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG CTTTGCAATCCCTTCTCCCATACAAAGTAAATATAATATATGCAACATATATGCCCATTCATCA GTGAAAAGCAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACCTTCGGTAAATAGCTACG

					TATCCTTCTCGCTTACTGGGGCCAAGGACTCTGGTCACCGTCTCCTCA
17.	VL of F6A	artificial	aa		QTVVTQEPSTLVSPGGTVTLTCSGSTGAVTSGYYPNWVQKPGQAPRGLIGGTKFLAPGTPARFS GSLGGKAAALTLGVPQPEDEAEYYCALWYSNRWVFGGTKLTVL
18.	VL of F6A	artificial	nt		CAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCATTG TGGTCTCTGACTGGGCTGTACATCTGGTACTACCCAAACTGGTCCACAAAAACCAAGGTC AGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCGGTACTCCTGCCAGATTCTCA GGTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTGCGTGGAGAACCAAACTGACTGTCC TA
19.	VH-P of F6A	artificial	aa		EVQLVESGGGLVQPGGSLKLSKAASGFTFNIIYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKSRFTISRDDSKNTAYLQMNNLKTEDTAVYICVRHGFNGNSYVFFAYWGQGTLLTVSS
20.	VH-P of F6A	artificial	nt		GAGTGGAGCTGCTCGAGTCTGGAGGAGGATTGGTGCACGCTGGAGGTCATTGAAACTCTCATG TGCAGCCCTCTGGATTCACTTCAATATCTACCGCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGGAAATGGTGTCTCGCATAGAAGTAAATATAATTAATGCAACATATATGCCGATTCA GTGAAAAGCAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACCTTCGGTAATAGCTACG TATCCTTCTTCGCTTACTGGGGCCAAGGACTCTGGTCACCGTCTCCTCA
21.	VL-P of F6A	artificial	aa		ELVVTQEPSTLVSPGGTVTLTCSGSTGAVTSGYYPNWVQKPGQAPRGLIGGTKFLAPGTPARFS GSLGGKAAALTLGVPQPEDEAEYYCALWYSNRWVFGGTKLTVL
22.	VL-P of F6A	artificial	nt		GAGCTCGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCATTG TGGTCTCTGACTGGGCTGTACATCTGGTACTACCCAAACTGGTCCACAAAAACCAAGGTC AGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCGGTACTCCTGCCAGATTCTCA GGTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTGCGTGGAGAACCAAACTGACTGTCC TA
23.	VH-VL of F6A	artificial	aa		EVQLVESGGGLVQPGGSLKLSKAASGFTFNIIYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKSRFTISRDDSKNTAYLQMNNLKTEDTAVYICVRHGFNGNSYVFFAYWGQGTLLTVSSGGGGS GGGSGGGGGSQTVVTQEPSTLVSPGGTVTLTCSGSTGAVTSGYYPNWVQKPGQAPRGLIGGTFK LAPGTPARFSCSLIGGKAAALTLGVPQPEDEAEYYCALWYSNRWVFGGTKLTVL

24.	VH-VL of F6A	artificial	nt	GAGTGCAGCTGGTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATATCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCAIAAGAAGTAAATATAATAATATGCAACATATATGCCGATTCA GTGAAAAAGCAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAA CTTGAATACTGAGGACACTGCCGTGTACTGTGTGAGACATGGAACTTCGGTAATAGCTACG TATCCTTCTTCGTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCAGGTGGTGGTGGTCT GGCGGGCGGCTCCGGTGGTGGTGGTCTCAGACTGTGTGACTCAGGAACCTTCACTCACCGT ATCACTGGTGAACAGTCACTCACTGTGGCTCTCGACTGGGCTGTACATCTGGCTACT ACCCAACTGGGTCCAAACAAAACAGGTCAAGCACCCCGTGGTCTAATAGTGGGACTAAGTTC CTCGCCCCGGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGCATGAGGCAGATATTACTGTCTCTATGTTACAGCAACCCGCTGGG TGTTCGGTGGAGCAACCAACTGACTGTCTTA
25.	VH-VL-P of F6A	artificial	aa	EVQLLESGGGLVQPGGSLKLSCAASGFTFNIIYAMNWVRQAPGKLEWVARIRSKYNNYATYYADS VKSRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFNSYVSFFAYWGQGLTVTVSSGGGGS GGGSGGGGSELVVTQEPSLTVSPGGTVTLTCSSTGAVTSGYYPNWVQKPGQAPRGLIGGIKF LAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCALWYSNRWVFGGKLTVL
26.	VH-VL-P of F6A	artificial	nt	GAGTGCAGCTGCTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATATCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCATAAGAAGTAAATATAATAATATGCAACATATATGCCGATTCA GTGAAAAAGCAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAA CTTGAATACTGAGGACACTGCCGTGTACTGTGGGCCAAGGACTCTGGTCAACCTCTCCTCAGGTGGTGGTCT TATCCTTCTTCGTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCAGGTGGTGGTGGTCT GGCGGGCGGCTCCGGTGGTGGTGGTCTGAGCTCGTTGTGACTCAGGAACCTTCACTCACCGT ATCACTGGTGAACAGTCACTCACTGTGGCTCTCGACTGGGCTGTACATCTGGCTACT ACCCAACTGGGTCCAAACAAAACAGGTCAAGCACCCCGTGGTCTAATAGTGGGACTAAGTTC CTCGCCCCGGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGCATGAGGCAGATATTACTGTCTCTATGTTACAGCAACCCGCTGGG TGTTCGGTGGAGCAACCAACTGACTGTCTTA
27.	CDR-L1 of H2C	artificial	aa	GSSTGAVTSGYYPN
28.	CDR-L2 of H2C	artificial	aa	CTKFLAP
29.	CDR-L3 of H2C	artificial	aa	ALWYSNRWV
30.	CDR-H1 of H2C	artificial	aa	KYAMN
31.	CDR-H2 of H2C	artificial	aa	RIRSKYNNYATYYADSVKD
32.	CDR-H3 of H2C	artificial	aa	HGNFNSYISWAY
33.	VH of H2C	artificial	aa	EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFNSYISWAYWGQGLTVTVSS

34.	VH of H2C	artificial	nt	GAGGTGCAGCTGGTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAAACCTCTCATG TGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCAIAAGAAGTAAATATAATAATATGCAACATATATTGCCGATTCA GTGAAAAGACAGGTTACACATCTCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTCAGGACACTGCCGTGTACTGTGTGAGACATGGGAACCTTCGGTAATAGCTACA TATCCTACTGGGCTTACTGGGGCCAAGGACTCTGGTCACCGTCTCCTCA
35.	VL of H2C	artificial	aa	QTVVTQEPSLTVSPGGTVTLTCCSSTGAVTSGYYPNWVQKPGQAPRGLIGGTKFLAPGTPARFS GSI.IGGKAAI.TI.SGVQPEDEAFYICAI.WYSNRWVFGGGTKI.TVL.
36.	VL of H2C	artificial	nt	CAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCACTTG TGGTCCCTCGACTGGGCTGTACATCTGGCTACTACCCAACTGGTCCACAAAAACCCAGGTC AGGCACCCCGTGGTCTAATAGGTGGACTAAGTTCCTCGCCCCCGGTACTCCTGCCAGATTCTCA GGTCCCTGCTTGAGGCAAGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATAATTACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTCCGGTGGAGGAACCAAACTGACTGTCC TA
37.	VH-P of H2C	artificial	aa	EVQLLESGGGLVQPGGSLKLSAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNLIKTEDTAVYYCVRHGFNGNSYISYWAYWGQTLTVSS
38.	VH-P of H2C	artificial	nt	GAGGTGCAGCTGCTCAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAAACCTCTCATG TGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCAIAAGAAGTAAATATAATAATATGCAACATATATTGCCGATTCA GTGAAAAGACAGGTTACACATCTCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTGTGTGAGACATGGGAACCTTCGGTAATAGCTACA TATCCTACTGGGCTTACTGGGGCCAAGGACTCTGGTCACCGTCTCCTCA
39.	VL-P of H2C	artificial	aa	ELVVTQEPSLTVSPGGTVTLTCCSSTGAVTSGYYPNWVQKPGQAPRGLIGGTKFLAPGTPARFS GSL.LGGKAAI.TI.SGVQPEDEAEYYICAI.WYSNRWVFGGGTKI.TVL.
40.	VL-P of H2C	artificial	nt	GAGCTCGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCACTTG TGGTCCCTCGACTGGGCTGTACATCTGGCTACTACCCAACTGGTCCACAAAAACCCAGGTC AGGCACCCCGTGGTCTAATAGGTGGACTAAGTTCCTCGCCCCCGGTACTCCTGCCAGATTCTCA GGTCCCTGCTTGAGGCAAGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATAATTACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTCCGGTGGAGGAACCAAACTGACTGTCC TA
41.	VH-VL of H2C	artificial	aa	EVQLVESGGGLVQPGGSLKLSAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNLIKTEDTAVYYCVRHGFNGNSYISYWAYWGQTLTVSSGGGGS GGGSGGGGSQTVVTQEPSLTVSPGGTVTLTCCSSTGAVTSGYYPNWVQKPGQAPRGLIGGTFK LAPGTPARH.SGSL.LGGKAAI.TI.SGVQPEDEAEYYICAI.WYSNRWVFGGGTKI.TVL
42.	VH-VL of H2C	artificial	nt	GAGGTGCAGCTGCTCAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAAACCTCTCATG TGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCAIAAGAAGTAAATATAATAATATGCAACATATATTGCCGATTCA

				GTGAAAGACAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACA TATCCTACTGGCTTACTGGGGCCAAGGACTCTGGTCAACCGTCTCCTCAGGTGGTGGTTCT GGCGGGGGCTCCGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCAACCGT ATCCTGTGTGAACAGTCACACTCACTGTGGCTCCTCGACTGGGCTGTTACATCTGGTACT ACCAAACTGGTCCAAACAAAACAGGTCAGGCACCCCGTGGTCTAATAGTGGGACTAAGTTC CTGCCCCGGTACTCCTGCCAGATTCTCAGGCTCCCTGCTGGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCCGCTGGG TGTTCCGTGGAGGAACCAACTGACTGTCTTA
43.	VH-VL-P of H2C	artificial	aa	EVQLLESGGGLVQPGGSLKLSKAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNLIKTEDTAVYYCVRHGFGNSYISYWAYWGQGLTVTVSSGGGGS GGGGGGGGSELVVTQEPSTVSPGGTVTLTSGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTFK LAPGTPARFSGSLGLGKAALTLSGVQPEDEAEYYCALWYSNRWVFGGTKLTVL
44.	VH-VL-P of H2C	artificial	nt	GAGTGCAGCTGCTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCATAGAAGTAATAATAATATGCAACATATTATGCCGATTCA GTGAAAGACAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCCTATCTACAAATGAACAA CTTGAAAACAGGACACTGCCGTGTACTGTGTGAGACATGGGAACCTCGGTAATAGCTACA TATCCTACTGGGCTTACTGGGGCCAAGGACTCTGGTCAACCGTCTCCTCAGGTGGTGGTGTCT GGCGGGGGCTCCGGTGGTGGTCTGAGCTCGTTGACTCAGGAACCTTCACTCAACCTCACCCT ATCACCTGGTGAACAGTCACACTCACTGTGGCTCCTCGACTGGGCTGTTACATCTGGCTACT ACCAAACTGGTCCAAACAAAACAGGTCAGGCACCCCGTGGTCTAATAGTGGGACTAAGTTC CTGCCCCGGTACTCCTGCCAGATTCTCAGGCTCCCTGCTGGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCCGCTGGG TGTTCCGTGGAGGAACCAACTGACTGTCTTA
45.	CDR-L1 of H1E	artificial	aa	GSSTGAVTSGYYPN
46.	CDR-L2 of H1E	artificial	aa	GTKFLAP
47.	CDR-L3 of H1E	artificial	aa	ALWYSNRWV
48.	CDR-H1 of H1E	artificial	aa	SYAMN
49.	CDR-H2 of H1E	artificial	aa	RIRSKYNNYATYYADSVKG
50.	CDR-H3 of H1E	artificial	aa	HGNFGNSYLSFWAY
51.	VH of H1E	artificial	aa	EVQLVDSGGGLEQPGGSLKLSKAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNLIKTEDTAVYYCVRHGFGNSYLSFWAYWGQGLTVTVSS
52.	VH of H1E	artificial	nt	GAGTGCAGCTGCTCGAGTCTGGAGGAGGATTGGAGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATTCGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCATAGAAGTAATAATAATATGCAACATATTATGCCGATTCA

					GTGAAAGGAGGTTACCATCTCCAGAGATGATTCAAAAAACAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACC TATCCTTCTGGCTTACTGGGCCAAGGACTCTGGTCACCGTCTCCTC
53.	VL of H1E	artificial	aa		QTVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTKFLAPGTPARFS GSLGGKAALTLGVPQPEDEAEYFCALWYSNRWVFGGTKLTVL
54.	VL of H1E	artificial	nt		CAGACTGTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCACTTG TGGTCCTCGACTGGGGCTGTACATCTGGCTACTACCCAAACTGGGTCCAAAAAACAGGTC AGGCAACCCGTGGTCTAATAGTGGGACTAAGTTCCTCGCCCCGGTACTCCTGCCAGATTCTCA GGTCCCCTGGCTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGTTACAGCAACCGCTGGGTGTTCCGTGGAGGAACCAAACTGACTGTCC TA
55.	VH-P of H1E	artificial	aa		EVQLVESGGGLEQPGGSLKLSCAASGFTFNSYAMNWWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYLSFWYWGQTLTVSS
56.	VH-P of H1E	artificial	nt		CAGGTGCAGCTGCTCAGTCTGGAGGAGGATTGGAGCAGCCTGGAGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATTCTGTAACCATGAATGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAGAAGTAATAATAATAATATGCAACATATTATGCCGATTCA GTGAAAGGAGGTTACCATCTCCAGAGATGATTCAAAAACAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACC TATCCTTCTGGCTTACTGGGCCAAGGACTCTGGTCACCGTCTCCTCA
57.	VL-P of H1E	artificial	aa		ELVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTKFLAPGTPARFS GSLGGKAALTLGVPQPEDEAEYFCALWYSNRWVFGGTKLTVL
58.	VL-P of H1E	artificial	nt		GAGTCGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCACTTG TGGTCCTCGACTGGGGCTGTACATCTGGCTACTACCCAAACTGGGTCCAAAAAACAGGTC AGGCAACCCGTGGTCTAATAGTGGGACTAAGTTCCTCGCCCCGGTACTCCTGCCAGATTCTCA GGTCCCCTGGCTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGTTACAGCAACCGCTGGGTGTTCCGTGGAGGAACCAAACTGACTGTCC TA
59.	VH-VL of H1E	artificial	aa		EVQLVESGGGLEQPGGSLKLSCAASGFTFNSYAMNWWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYLSFWYWGQTLTVSSGGGGS GGGSGGGGSGTQVVTQEPSTLTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTFK LAPGTPARFSGSLGKKAALTLGVPQPEDEAEYFCALWYSNRWVFGGTKLTVL
60.	VH-VL of H1E	artificial	nt		GAGTCAGCTGGTCTGAGTCTGGAGGAGGATTGGAGCAGCCTGGAGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATTCTGTAACCATGAATGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGGTCTCGCATAGAAGTAATAATAATAATATGCAACATATTATGCCGATTCA GTGAAAGGAGGTTTACCATCTCCAGAGATGATTCAAAAACAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACC TATCCTTCTGGCTTACTGGGCCAAGGACTCTGGTCAACCGTCTCCTCAGGTGGTGGTGTCT

				GGGGGGGGCTCCGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGT ATCACCCTGGTGAAACAGTCACACTCACTTGTGGTCTCTCGACTGGGCTGTACATCTGGCTACT ACCCAAACTGGTCCAAACAAAACCAGGTCAGGCACCCCGTGGTCTAATAGGTGGACTAAGTTC CTCGCCCCGGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCCCT CTCAGGGGTACAGCCAGAGGATGAGGCAGAAATATTACTGTGCTCTATGTTACAGCAACCGCTGGG TGTTGGTGGAGGAACCAACTGACTGTCTTA
61.	VH-VL-P of H1E	artificial	aa	EVQLVESGGLEQPGGSLKLSCAASGFTFNSYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYIQMNNIKTEDTAVYYCVRHGNGFSYI.SFWAYWGQGTI.VTVSSGGGGS GGGGGGGSELVVTQEPSLTVSPGGTVTLTCSSTGAVTSGYYPNVVQKPGQAPRGLIGGTRK LAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCALWYSNRWVEGGGKLTIVL
62.	VH-VL-P of H1E	artificial	nt	GAGGTGCAGCTGCTCGAGTCTGGAGGAGGATTGGAGCAGCCTGGAGGGTCATTGAAACTCTCATG TGAGCCCTCGGATTCACTTCAATTCTGACCGCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGATAAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAGGAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGTAATAGCTACC TATCCTTCTGGCTTACTGGGGCCAAGGACTCTGGTCAACCGTCTCCTCAGGTGGTGGTGTCT GGGGGGGGCTCCGGTGGTGGTCTGAGCTCGTGTGACTCAGGAACCTTCACTCACCGT ATCACCTGGTGAAACAGTCACACTCACTTGTGGTCTCTCGACTGGGCTGTACATCTGGCTACT ACCCAAACTGGTCCAAACAAAACCAGGTCAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTC CTCGCCCGGCTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCCCT CTCAGGGGTACAGCCAGAGGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCGCTGGG TGTTGGTGGAGGAACCAACTGACTGTCTTA
63.	CDR-L1 of G4H	artificial	aa	GSSIGAVISGYYPN
64.	CDR-L2 of G4H	artificial	aa	GTKFLAP
65.	CDR-L3 of G4H	artificial	aa	ALWYSNRWV
66.	CDR-H1 of G4H	artificial	aa	RYAMN
67.	CDR-H2 of G4H	artificial	aa	RIRSKYNNYATYYADSVKG
68.	CDR-H3 of G4H	artificial	aa	HGNFGNSYLSYFAY
69.	VH of G4H	artificial	aa	EVQLVESGGLEQPGGSLKLSCAASGFTFNRYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYIQMNNIKTEDTAVYYCVRHGNGFSYLSYFAYWGQGTI.VTVSS
70.	VH of G4H	artificial	nt	GAGTGCAGCTGCTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGAGCCCTCGGATTCACTTCAATCGCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTTGCTCGCATAAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAGGAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACCTTCGGTAATAGCTACT TATCCTACTTCGCTTACTGGGGCCAAGGACTCTGGTCAACCGTCTCTCTCA

71.	VL of G4H	artificial	aa	QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTKFLAPGTPARES GSLGGKAAALTLGVPQPEDEAEYYCALWYSNRWVFGGKLTIVL
72.	VL of G4H	artificial	nt	CAGACTGTTGTGACTCAGGAACCTTCACCTACCGTATCACCTGGTGGACAGTCACACTCACTTG TGGCTCCTCGACTGGGCTGTACATCTGGTACTACCCAAACTGGTCCAAACAAACAGGTC AGGCACCCGCTGCTAATAGGTGGACTAAGTTCCTCGCCCCCGTACTCTGCCAGATTCTCA GGTCCCTGCTTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTCCGGTGGAGAACCAAACTGACTGTCC TA
73.	VH-P of G4H	artificial	aa	EVQLLESGGGLVQPGGSLKLSAASGFTFNRYAMNWRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFGNSYLSYFAYWGQGLTVTVSS
74.	VH-P of G4H	artificial	nt	GAGGTGAGCTGCTGAGTCTGGAGGAGGATTGGTGCAGCTGGAGGCTCATTTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATCGCTACGCCATGAACCTGGTCCGCCAGGTCAGGAAAGG GTTTGGAAATGGGTGCTCGATAAAGAAAGTAAATATAATAATATGCAACATATATGCCGATTCA GTGAAAGGGAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACCTTCGGTAATAGCTACT TATCCTACTTCGCTTACTGGGGCCAGGACTCTGGTCACCGTCTCCTCA
75.	VL-P of G4H	artificial	aa	ELVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTKFLAPGTPARES GSLGGKAAALTLGVPQPEDEAEYYCALWYSNRWVFGGKLTIVL
76.	VL-P of G4H	artificial	nt	GAGCTCGTTGTGACTCAGGAACCTTCACCTACCGTATCACCTGGTGGAAACAGTCACACTCACTTG TGGCTCCTCGACTGGGCTGTACATCTGGTACTACCCAAACTGGTCCAAACAAACAGGTC AGGCACCCGCTGCTAATAGGTGGACTAAGTTCCTCGCCCCCGTACTCTGCCAGATTCTCA GGTCCCTGCTTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGCTCTATGGTACAGCAACCGCTGGGTGTTCCGGTGGAGAACCAAACTGACTGTCC TA
77.	VH-VL of G4H	artificial	aa	EVQLVESGGGLVQPGGSLKLSAASGFTFNRYAMNWRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFGNSYLSYFAYWGQGLTVTVSSGGGGS GGGGGGGGSTVVTQEPSTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTKF LAPGTPARFSGSLIGGKAAALTLGVPQPEDEAEYYCALWYSNRWVFGGKLTIVL
78.	VH-VL of G4H	artificial	nt	GAGGTGAGCTGCTGAGTCTGGAGGAGGATTGGTGCAGCTGGAGGCTCATTTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATCGCTACGCCATGAACCTGGTCCGCCAGGTCAGGAAAGG GTTTGGAAATGGGTGCTCGATAAAGAAAGTAAATATAATAATATGCAACATATATGCCGATTCA GTGAAAGGGAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACCTTCGGTAATAGCTACT TAATCCACTTCGCTTACTGGGGCCAGGACTCTGGTCAACCTCTCCTCAGGTGGTGGTTCCT GGGGGGGGCTCCGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACCTCACCGT ATCACCTGGTGGAAACAGTCACACTCACTTGTGGCTCCTCGACTGGGCTGTTACATCTGGCTACT ACCAAACTGGGTCCCAACAAAAACAGGTCAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTC

				CTGCCCCGGTACTCCTGCCAGATTCTCAGGCTCCCTGCTTGGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACGCCAGAGGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCCGCTGGG TGTTCCGTGAGGAACCAACTGACTGTCTCTA
79.	VH-VL-P of G4H	artificial	aa	EVQLLESGGGLVQPGSLKLSKAASGFTFNRYAMNWVRQAPGKLEWVARIRSKYNNYATYYADS VKRFTISRDDSKNTAYLQMNILKTEDTAVYYCVRHGNGNSYLSYFAYWGQGLTVTVSSGGGGS GGGSGGGSELVVTQEPSTLVSPGGTVTLTCSSTGAVTSGYYPNWWQKPGQAPRGLIGGTFK LAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCALWYSNRWVFGGGLKLTVL
80.	VH-VL-P of G4H	artificial	nt	GAGTGCAGCTGCTCAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGCTATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATCGCTACGCCATGAACCTGGGTCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAGAAGTAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAGGGAGGTTACCATCTCCAGAGATGATCAAAAACACTGCCTATCTACAAATGAACAA CTTGAATACTGAGCACACTCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACT TATCCTACTTCGTTACTGGGCCAAGGACTCTGTGCACCTCTCCTCAGGTGGTGGTTCT GGCGGGGGCTCCGGTGGTGGTGTGCTGAGCTCGTGTGACTCAGGAACCTTCACTCACCGT ATCAGCTGGTGAACAGTCACACTACTTGTGGTCTCTCGACTGGGCTGTACATCTGGCTACT ACCCAACTGGGTCCAAACAAAACAGGTCAGGCACCCCGTGGTCTAATAGTGGGACTAAGTTC CTGCCCCCGGTACTCTCTCCAGATTCTCAGGCTCCCTGCTGGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCCGCTGGG TGTTCCGTGAGGAAACCAAACTGACTGTCTCTA
81.	CDR-L1 of A2J	artificial	aa	RSSTGAVTSGYYPN
82.	CDR-L2 of A2J	artificial	aa	ATDMRPS
83.	CDR-L3 of A2J	artificial	aa	ALWYSNRWY
84.	CDR-H1 of A2J	artificial	aa	VYAMN
85.	CDR-H2 of A2J	artificial	aa	RIRSKYNNYATYYADSVKK
86.	CDR-H3 of A2J	artificial	aa	HGNFGNSYLSWWAY
87.	VH of A2J	artificial	aa	EVQLVESGGGLVQPGSLKLSKAASGFTFNRYAMNWVRQAPGKLEWVARIRSKYNNYATYYADS VKRFTISRDDSKNTAYLQMNILKTEDTAVYYCVRHGNGNSYLSWWAYWGQGLTVTVSS
88.	VH of A2J	artificial	nt	GAGTGCAGCTGGTTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGCTCATGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATGTCTACGCCATGAACCTGGGTCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAGAAGTAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAAAGAGGTTACCATCTCCAGAGATGATCAAAAACACTGCCTATCTACAAATGAACAA CTTGAATACTGAGCACACTCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACT TATCCTGGTGGCTTACTGGGCCAAGGACTCTGTGCACCTCTCCTCA
89.	VL of A2J	artificial	aa	QTVVTQEPSTLVSPGGTVTLTCSRSTGAVTSGYYPNWWQKPGQAPRGLIGATDMRPSGTPARFS GSLLGKKAALTLSGVQPEDEAEYYCALWYSNRWVFGGGLKLTVL
90.	VL of A2J	artificial	nt	CAGACTGTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCACTTG

				TCGCTCCTCGACTGGGGCTGTTACATCTGGCTACTACCCAAACTGGGTCCAACAAAAACCAGGTC AGGCACCCCGTGGTCTAATAGGTGCCACTGACATGAGGCCCTCTGGTACTCTGCCAGATTCTCA GGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGTTACAGCAACCGCTGGGTGTTCCGGTGGAGGAACCAAACTGACTGTCC TA
91.	VH-P of A2J	artificial	aa	EVQLVESGGGLVQPGGSLKLSKAASGFTFNVYAMNWVRQAPGKLEWVARIRSKYNNYATYYADS VKRFTISRDDSKNTAYLQMNNLKTEDTAVYVCVRHGFNGNSYLSWYWGQGLTVTVSS
92.	VH-P of A2J	artificial	nt	GAGTGCAGCTGCTGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGCTCATTGAAAACCTCTCATG TGCAGCCTCTGGATTCACTTCAATGTCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAGAAGTAAATATAATTAATCAACATATTATGCCGATTCA GTGAAAAAGAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCCTATCTACAAATGAACAA CTTGAATACTGAGCACACTGCCGTGTAATACTGTGTGAGACATGGAACTTCGGTAATAGCTACT TATCCTGGTGGCTTACTGGGGCAAGGACTCTGGTCACCGTCTCCTCA
93.	VL-P of A2J	artificial	aa	ELVYTQEPSTLVSPGGTVTLTCRSSTGAVTSGYYPNWWQKPGQAPRGLIGATDMRPSGTPARFS GSLGGKKAALTLSGVQPEDEAEYYCALWYSNRWVFGGTGKLTVL
94.	VL-P of A2J	artificial	nt	GAGTCGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCATTTG TCGTCCTCGACTGGGGCTGTTACATCTGGCTACTACCCAAACTGGTCCAACAAAAACCAGGTC AGGCACCCCGTGGTCTAATAGGTGCCACTGACATGAGGCCCTCTGGTACTCTGCCAGATTCTCA GGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGTTACAGCAACCGCTGGGTGTTCCGGTGGAGGAACCAAACTGACTGTCC TA
95.	VH-VL of A2J	artificial	aa	EVQLVESGGGLVQPGGSLKLSKAASGFTFNVYAMNWVRQAPGKLEWVARIRSKYNNYATYYADS VKRFTISRDDSKNTAYLQMNNLKTEDTAVYVCVRHGFNGNSYLSWYWGQGLTVTVSSGGGGS GGGSGGGGSQTVVTQEPSTLVSPGGTVTLTCRSSTGAVTSGYYPNWWQKPGQAPRGLIGATDM RPSGTPARFSGSLGGKKAALTLSGVQPEDEAEYYCALWYSNRWVFGGTGKLTVL
96.	VH-VL of A2J	artificial	nt	GAGTGCAGCTGCTGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGCTCATTGAAAACCTCTCATG TGCAGCCTCTGGATTCACTTCAATGTCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATAAGGTTGCTCGCATAGAAGTAAATATAATTAATTAATCAACATATTATGCCGATTCA GTGAAAAAGAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCCTATCTACAAATGAACAA CTTGAATACTCAGGACACTGCCGTGTAATACTGTGTGAGACATGGAACTTCGGTAATAGCTACT TATCCTGGTGGCTTACTGGGGCAAGGACTCTGGTCAACCGTCTCCTCAGGTGGTGGTGGTCT GGCGGGCGGCTCCGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGT ATCACCTGGTGAACAGTCACACTCACTGTGCTCTCGACTGGGCTGTACATCTGGCTACT ACCCAAACTGSGTCCAAACAAAAACAGGTCAGGCACCCCGTGGTCTAATAGGTGCCACTGACATG AGGCCCTCTGGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCGCTGGG TGTTCCGGTGAGGAACCAAACTGACTGTCTCTA

97.	VH-VL-P of A2J	artificial	aa	EVQLVESGGGLVQPGGSLKLSKAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGNSYLSWYAYWGQGLTVTVSSGGGGS GGGSGGGSELVVTQEPSTLVSPGGTVTLTCSRSTGAVTSGYYPNWVQKPGQAPRGLIGATDM RPSGTPARFSGSLGGAALTLSGVQPEDEAEFYCALWYSNRWVFGGGLKLTVL
98.	VH-VL-P of A2J	artificial	nt	GAGTGCAGCTGCTCAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAAACCTCTCATG TGCAGCCTCTGGATTCACTTCAATGTCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAGAAGTAAATATAATATATGCAACATATTATGCCGATTCA GTGAAAAGAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCATCTACAAATGAACAA CTTGAAAACCTGAGGACACTGCCGTGTAATACTGTGTGAGACATGGGAACCTCGGTAAATAGCTACT TATCCTGGTGGCTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCAGTGGTGGTGGTCTCT GGGGGGGGCTCCGGTGGTGGTCTGAGCTCGTGTGACTCAGGAACCTTCACTCACCGT ATCACCTGGTGGAAACGTCACACTCACTGTGCTCCTCGACTGGGCTGTACATCTGGCTACT ACCAAACTGGGTCCAAACAAAACCAAGGTCAAGCACCCCGTGGTCTAATAGTGCCACTGACATG AGGCCCTCTGTACTCTCTGCCAGATTCTCAGGCTCCCTGCTGGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCGCTGGG TGTTCCGTGGAGAACCAAACTGACTGTCTCTA
99.	CDR-L1 of E1L	artificial	aa	GSSTGAVTSGYYPN
100.	CDR-L2 of E1L	artificial	aa	GTKELAP
101.	CDR-L3 of E1L	artificial	aa	AIWYSNRWV
102.	CDR-H1 of E1L	artificial	aa	KYAMN
103.	CDR-H2 of E1L	artificial	aa	RTRSKYNNYATYYADSVKS
104.	CDR-H3 of E1L	artificial	aa	HGNFGNSYTSYYAY
105.	VH of E1L	artificial	aa	EVQLVESGGGLVQPGGSLKLSKAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGNSYTSYYAYWGQGLTVTVSS
106.	VH of E1L	artificial	nt	GAGTGCAGCTGCTCAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAAACCTCTCATG TGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAGAAGTAAATATAATATATGCAACATATTATGCCGATTCA GTGAAATCGAGGTTACCATCTCCAGAGATGATTCAAAAAACACTGCCATCTACAAATGAACAA CTTGAAAACCTGAGGACACTGCCGTGTAATACTGTGTGAGACATGGGAACCTCGGTAAATAGCTACA CATCTACTACGCTTACTGGGGCCAAGGACTCTGGTCAACCTCTCTCTCA
107.	VL of E1L	artificial	aa	QTVVTQEPSTLVSPGGTVTLTCSSTGAVTSGYYPNWVQKPGQAPRGLIGGTFKFLAPGTPARFS GSLGGKAALTLSGVQPEDEAEFYCALWYSNRWVFGGGLKLTVL
108.	VL of E1L	artificial	nt	CAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCACTTG TGGCTCCTCGACTGGGGCTGTACATCTGGCTACTACCCAAACTGGGTCCAAACAAAACAGGTC AGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCTCGCCCCCGTACTCTCTGCCAGATTCTCA GGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA

109.	VH-P of E1L	artificial	aa	ATATTACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTCCGGTGGAGGAACCAAACTGACTGTCC TA
110.	VH-P of E1L	artificial	nt	EVQLLESGGGLVQPGGSLKLSAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKSRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYTSYYAWGQGLTVTVSS GAGTGCAGCTGCTCGAGTCTCGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAGAAGATAATAATAATAATAATATGCAACATATTATGCCGATTCA GTGAATCGAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCCTATCTACAAATGAACAA CTTGAAAACCTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACA CATCTACTACGCTTACTGGGGCCCAAGGACTCTGGTCACCGTCTCCTCA
111.	VL-P of E1L	artificial	aa	ELVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTFKFLAPGTPARFS GSLLGKAAALTLSGVQPEDEAEYYCALWYSNRWVFGGTKLTVL
112.	VL-P of E1L	artificial	nt	GAGTCGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCACTTG TGGCTCCTCGACTGGGCTGTACATCTGGCTACTACCAAACTGGTCCAAACAAACCCAGGTC AGGACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCGGTACTCCTGCCAGATTCTCA GGCTCCTGTCTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTCCGGTGGAGGAACCAAACTGACTGTCC TA
113.	VH-VL of E1L	artificial	aa	FVQIVFSGGGLVQPGGSLKLSAASGFTFNKYAMNWVRQAPGKGI,FWVARI RSKYNNYATYYADS VKSRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYTSYYAWGQGLTVTVSSGGGS GGGSGGGGQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTFK LAPGTPARFSGSLLGKAAALTLSGVQPEDEAEYYCALWYSNRWVFGGTKLTVL
114.	VH-VL of E1L	artificial	nt	GAGTGCAGCTGGTCTGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATAAGTACCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAGAAGATAATAATAATAATAATATGCAACATATTATGCCGATTCA GTGAATCGAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCCTATCTACAAATGAACAA CTTGAAAACCTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACA CATCCTACTACGCTTACTGGGGCCCAAGGACTCTGGTCACCGTCTCCTCAGGTGGTGGTGTCT GGCGGGGGGCTCCGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGT ATCAGCTGGTGAACAGTCACACTCACTGTGGTCTCGACTGGGCTGTTACATCTGGCTACT ACCAAACTGGTCCAAACAAAACAGGTCAGGACCCCGTGGTCTAATAGTGGGACTAAGTTC CTGCCCCCGGTACTCTGCCAGATTCTCAGGCTCCCTGCTGGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGGATGAGGCAGATAATTAATGTGCTCTATGGTACAGCAACCCGCTGGG TGTTCCGTTGAGGAACCAAACTGACTGTCTCTA
115.	VH-VL-P of E1L	artificial	aa	EVQLLESGGGLVQPGGSLKLSAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKSRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYTSYYAWGQGLTVTVSSGGGS GGGSGGGGSELVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGTFK

116.	VH-VL-P of E1L	artificial	nt	LAPGTPARFSGSLGGKAAALTLGSGVQPEDEAEYYCALWYSNRWVFGGKLTIVL GAGGTGAGCTGCTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAACCTGGGTCGGCAGGCTCCAGGAAAGG GTTTGAATGGTGTGCTCGCATAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAATCGAGGTTACATCTCAGAGATGATCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAATACTAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACA CATCTACTACGTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCAGGTGGTGGTCTCT GGCGGGCGGCTCCGGTGGTGGTCTGAGCTCGTTGACTCAGGAACCTTCACTCACCCGT ATCACTGGTGAACAGTCACTCACTGTGGCTCCTCGACTGGGCTGTTACATCTGGCTACT ACCCAACTGGGTCCAAACAAAACAGGTCAAGCACCCCGTGGTCTAATAGTGGGACTAAGTTC CTGCCCCCGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGCATGAGGCAGATATATTACTGTCTATGTTACAGCAACCCGCTGGG TGTTGGGTGAGGAACCAAACTGACTGTCTCTA
117.	CDR-L1 of E2M	artificial	aa	RSSTGAVTSGYYPN
118.	CDR-L2 of E2M	artificial	aa	ATDMRPS
119.	CDR-L3 of E2M	artificial	aa	ALWYSNRWV
120.	CDR-H1 of E2M	artificial	aa	GYAMN
121.	CDR-H2 of E2M	artificial	aa	RIRSKYNNYATYYADSVKE
122.	CDR-H3 of E2M	artificial	aa	HRNFGNSYLSWFAY
123.	VH of E2M	artificial	aa	EVQLVESGGGLVQPGGSLKLSCAASGFTFNGYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKERFTISRDDSKNTAYLQMNILKTEDTAVYYCVRHRNFGNSYLSWFAYWGQTLTVSS
124.	VH of E2M	artificial	nt	GAGGTGAGCTGCTGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATGGCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTGCTCGCATAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAGAGAGGTTACATCTCAGAGATGATCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAATACTAGGACACTGCCGTGTACTACTGTGTGAGACATAGGAACCTTCGGTAATAGCTACT TATCCTGGTTCGCTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCA
125.	VL of E2M	artificial	aa	QTVVTQEPSTLVSPGGTVVLTCSRSTGAVTSGYYPNWVQKPGQAPRGLIGATDMRPSGTPARFS GSLGGKAAALTLGSGVQPEDEAEYYCALWYSNRWVFGGKLTIVL
126.	VL of E2M	artificial	nt	CAGACTGTTGTGACTCAGGAACCTTCACTCAACCTGATCACCTGGTGGAGACAGTCACACTCACTTG TCGCTCTCGACTGGGCTGTACATCTGGTACTACCCAACTGGTCCACAAACAAACCCAGGTC AGGCACCCCGTGGTCTAATAGTGTCCACTGACATGAGGCCCTCTGTACTCTCTGCCAGATTCTCA GGTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGGTACAGCAACCGCTGGGTGTTCTGGTGGAGGAACCAAACTGACTGTCC TA
127.	VH-P of E2M	artificial	aa	EVQLLESGGGLVQPGGSLKLSCAASGFTFNGYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS

128.	VH-P of E2M	artificial	nt	VKERFTISRDDSKNTAYLQMNLLKTEDTAVYYCVHRNFGNSYLSWFAYWGQTLTVVSS GAGGTGAGCTGCTCGAGTCTGGAGGAGGATTGGTGACGCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATGGCTACGCCATGAACCTGGGTCGGCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCATAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAGAGAGGTTACATCTCAGAGATGATCAAAAAACACTGCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATAGGAACCTTCGGTAATAGCTACT TATCTGTTCTGCTTACTGGGGCAAGGACTCTGGTCACCGTCTCTCTCA
129.	VL-P of E2M	artificial	aa	ELVVTQEPSTLVSPGGTVTLTCSRSTGAVTSGYYPNWVQKPGQAPRGLIGATDMRPSGTPAREF GSLGGKAAALTLSGVQPEDEAEYYCALWYSNRWVFGGKLTVL
130.	VL-P of E2M	artificial	nt	GAGTCGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGACAGTCACACTCACTTG TCGTCCTCGACTGGGCTGTACATCTGGCTACTACCCAACTGGTCCACAAAACACAGGTC AGGCACCCGTTGTTAATAGGTGCCACTGACATGAGGCCCTCTGTACTCTGCCAGATTCTCA GGTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGGTACAGCAACCGTGGGTGTTTCGGTGGAGGAACCAAACTGACTGTCC TA
131.	VH-VL of E2M	artificial	aa	EVQLVESGGGLVQPGGSLKLSAASGFTFNGYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKERFTISRDDSKNTAYLQMNLLKTEDTAVYYCVHRNFGNSYLSWFAYWGQTLTVVSSGGGGS GGGSGGGGSGTVVTQEPSTLVSPGGTVTLTCSRSTGAVTSGYYPNWVQKPGQAPRGLIGATDM RPSGTPARFSGSLGGKAAALTLSGVQPEDEAEYYCALWYSNRWVFGGKLTVL
132.	VH-VL of E2M	artificial	nt	GAGGTGAGCTGCTCGAGTCTGGAGGAGGATTGGTGACGCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATGGCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTTGCTCGCATAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAGAGAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATAGGAACCTTCGGTAATAGCTACT TATCCTGTTCTGTTACTGGGGCCAAAGGACTCTGGTCACCGTCTCCTCAGGTGGTGGTGTCT GGCGGCGCGCTCCGGTGGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGT ATCACCTGGTGAACAGTCACACTCACTGTGCTCTCGACTGGGCTGTACATCTGGCTACT ACCCAAACTGGTCCAAACAAAACAGGTACGGCACCCCGTGGTCTAATAGGTGCCACTGACATG AGGCCCTCTGGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCGCTGGG TGTTCTGGTGGAGAACCAAACTGACTGTCTTA
133.	VH-VL-P of E2M	artificial	aa	EVQLVESGGGLVQPGGSLKLSAASGFTFNGYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKERFTISRDDSKNTAYLQMNLLKTEDTAVYYCVHRNFGNSYLSWFAYWGQTLTVVSSGGGGS GGGSGGGGSELVVTQEPSTLVSPGGTVTLTCSRSTGAVTSGYYPNWVQKPGQAPRGLIGATDM RPSGTPARFSGSLGGKAAALTLSGVQPEDEAEYYCALWYSNRWVFGGKLTVL
134.	VH-VL-P of E2M	artificial	nt	GAGGTGAGCTGCTCGAGTCTGGAGGAGGATTGGTGACGCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATGGCTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG

				GTTTGAATGGTTGCTCGCATAAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAGAGAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCCTATCTACAAATGAACAA CTTGAAAAC TGAGACACTGCCGTGTACTACTGTGTGAGACATAGGAACCTCGGTAATAGCTACT TATCCTGGTTCGCTTACTTGGGGCCAAGGACTCTGGTCACCGTCTCCTCAGGTGGTGGTTCT GGCGGGCGGCTCCGGTGGTGGTCTGAGCTCGTTGTGACTCAGGAACCTTCACTCACCGT ATCACCTGGTGAACAGTCACACTCACTGTCTGCTCCTCGACTGGGCTGTTACATCTGGCTACT ACCCAAACTGGGTCCAA CAAAACCAGGTCAGGCACCCCGTGGTCTAATAGTGCCACTGACATG AGGCCCTCTGGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGGAGGCAAGGTCGCCCTCACCTT CTCAGGGGTACGCCAGAGATGAGGCAGAAATATTACTGTGCTCTATGGTACAGCAACCCGCTGGG TGTTCCGTTGGAGGAACCAAACTGACTGTCTTA
135.	CDR-L1 of F70	artificial	aa	GSSTGAVTSGYYPN
136.	CDR-L2 of F70	artificial	aa	GTKFLAP
137.	CDR-L3 of F70	artificial	aa	ALWYSNRWV
138.	CDR-H1 of F70	artificial	aa	VYAMN
139.	CDR-H2 of F70	artificial	aa	RIRSKYNNYATYYADSVKK
140.	CDR-H3 of F70	artificial	aa	HGNFGNSYISWVAY
141.	VH of F70	artificial	aa	EVQLVESGGGLVOPGGS LKLSCAASGFTFNVYAMNWVRQAPGKLEWVARI RSKYNNYATYYADS VKRRTISRDDSKNTAYLQMNILKTEDTAVYICVRHGNFGNSYISWVAYWGQGLTVVSS
142.	VH of F70	artificial	nt	GAGGTGAGCTGGTCGAGTCTGGAGGAGGATTGGTGACGCTGGAGGTCATTGAAACTCTCATG TGACCCCTCTGGATTCACTTCAATGTGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTTGCTCGCATAAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAAAGAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCCTATCTACAAATGAACAA CTTGAAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACCTCGGTAATAGCTACA TATCCTGGTGGGCTTACTGGGCGCAAGGACTCTGGTCACCGTCTCCTCA
143.	VL of F70	artificial	aa	QTVVTFEPSTLVSPGGTTLTTCGSSSTGAVTSGYYPNWWQKPGQAPRGLIGGTKFLAPGTPARFS GSLGKGKAAITLSGVQPEDEAEYICALWYSNRWVFGGTKLTVL
144.	VL of F70	artificial	nt	CAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCATTG TGGCTCTCGACTGGGCTGTACATCTGGCTACTACCCAAACTGGTCCCAACAAAACAGGTC AGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCCTCGCCCCCGTACTCCTGCCAGATTCTCA GGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGCTCTATGTTACAGCAACCGCTGGGTGTTGGTGGAGGAACCAAACTGACTGTCC TA
145.	VH-P of F70	artificial	aa	EVQLVESGGGLVOPGGS LKLSCAASGFTFNVYAMNWVRQAPGKLEWVARI RSKYNNYATYYADS VKRRTISRDDSKNTAYLQMNILKTEDTAVYICVRHGNFGNSYISWVAYWGQGLTVVSS
146.	VH-P of F70	artificial	nt	GAGGTGAGCTGCTCGAGTCTGGAGGAGGATTGGTGACGCTGGAGGTCATTGAAACTCTCATG TGCAGCCCTCTGGATTCACTTCAATGTGTACGCCATGAACCTGGGTCCGCCAGGCTCCAGGAAAGG

147.	VL-P of F70	artificial	aa	GTTTGAATGGTTGTCGCATAAGAAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAAAGAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCATCTACAAATGAACAA CTTGAAAAACTGAGGACACTGCCGTGTACTGTGTGAGACATGGAACCTCGGTAAATAGCTACA TATCCTGGTGGCTTACTGGGGCCAAGGACTCTGGTCACCGTCTCCTCA ELVVTQEPSTLVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGKFLAPGTPARES GSLGGKAALTLGVPQPEDEAEYYCALWYSNRWVFGGKLTVL
148.	VL-P of F70	artificial	nt	GAGTCGTTGTCACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACTCACTTG TGGTCTCTGACTGGGCTGTACATCTGGCTACTACCCAAACTGGTCCAAAAACCAAGGTC AGGCAACCCGTGGTCTAATAGGTGGACTAAGTTCCTCGCCCCGGTACTCCTGCCAGATTCTCA GGTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCAGAGGATGAGGCAGA ATATTACTGTCTCTATGTTACAGCAACCGCTGGGTGTCGGTGGAGGAACCAAACTGACTGTCC TA
149.	VH-VL of F70	artificial	aa	EVQLVESGGGLVQPGGSLKLSKAASGFTFNVYAMNWRQAPGKGLEWVARIRSKYNNYATYYADS VKRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFNSYISWWAYWGQGLTVTVSSGGGS GGGGGGGSGTVVTQEPSTLVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGKXF LAPGTPARESGSLGGKAALTLGVPQPEDEAEYYCALWYSNRWVFGGKLTVL
150.	VH-VL of F70	artificial	nt	GAGTGCAGCTGGTCGAGTCTGGAGGAGGATTGGTCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATGTGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTTGCTCGAIAAGAAAGTAAATATAATATGCAACATATTATGCCGATTCA GTGAAAAAGAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCATCTACAAATGAACAA CTGAAAAACTGAGGACACTGCCGTGTACTGTGTGAGACATGGAACTTCGGTAATAGCTACA TATCCTGGTGGCTTACTGGGGCCAAGGACTCTGGTCACTCCCTCAGTGGTGGTGGTGGTCT GGCGGGGGCTCCGGTGGTGGTGGTCTCAGACTGTGTGACTCAGGAACCTTCACTCACCGT ATCACCCTGGTGAACAGTCACTCACTTGTGGCTCTCGACTGGGCTGTACATCTGGCTACT ACCCAACTGGTCCAAACAAACAGGTCAGGCAACCCCTGGTCTAATAGTGGGACTAAGTTC CTCGCCCCGGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGGATGAGGCAGATATTACTGTGCTCTATGTTACAGCAACCGCTGGG TGTTCGGTGGAGAACCAAACTGACTGTCTCTA
151.	VH-VL-P of F70	artificial	aa	EVQLVESGGGLVQPGGSLKLSKAASGFTFNVYAMNWRQAPGKGLEWVARIRSKYNNYATYYADS VKRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFNSYISWWAYWGQGLTVTVSSGGGS GGGGGGGSELVVTQEPSTLVSPGGTVTLTCGSSTGAVTSGYYPNWWQKPGQAPRGLIGGKXF LAPGTPARESGSLGGKAALTLGVPQPEDEAEYYCALWYSNRWVFGGKLTVL
152.	VH-VL-P of F70	artificial	nt	GAGTGCAGCTGGTCGAGTCTGGAGGAGGATTGGTCAGCCTGGAGGGTCATTGAAACTCTCATG TGCAGCCTCTGGATTCACTTCAATGTGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTTGCTCGCATAAGAAAGTAAATATAATATGCAACATATTATGCCGATTCA GTGAAAAAGAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCATCTACAAATGAACAA CTTGAACAACTGAGGACACTGCCGTGTACTGTGTGAGACATGGAACTTCGGTAATAGCTACA

				TATCCTGGTGGGCTTACTGGGGCCAAAGGACTCTGGTCACCGTCTCCTCAGGTGGTGGTTCT GGGGGGCGGCTCCGGTGGTGGTCTGAGCTCGTTGTGACTCAGGAACCTTCACTCACCGT ATCACCTGGTGAACAGTCACACTCACTTGTGGTCTCTGACTGGGCTGTACATCTGGCTACT ACCAAACTGGTCCAAACAAAAACAGGTCAAGCACCCCGTGGTCTAATAGGTGGACTAAGTTC CTGCCCGCCGCTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCCAGAGGATGAGGCAGATATTAATCTGTGCTCTATGGTACAGCAACCGCTGGG TGTTGGTGGAGGAACCAAACTGACTGTCTCTA
153.	CDR-L1 of F12Q	artificial	aa	GSSTGAVTSGNYPN
154.	CDR-L2 of F12Q	artificial	aa	GTKFLAP
155.	CDR-L3 of F12Q	artificial	aa	VLWYSNRWV
156.	CDR-H1 of F12Q	artificial	aa	SYAMN
157.	CDR-H2 of F12Q	artificial	aa	RIRSKYNNYATYYADSVKG
158.	CDR-H3 of F12Q	artificial	aa	HGNEGNSYVSWWAY
159.	VH of F12Q	artificial	aa	EVQLVESGGGLVQPGGSLKLSCAASGFTFNSYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYVSWWAYWGQGLTVTVSS
160.	VH of F12Q	artificial	nt	GAGGTGCAGCTGGTGCAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCACTTGAACCTCTCATG TGACGCCCTCTGGATTCACTTCAATAGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGTGTCTCGCATAAAGAAATAATAATAATATGCAACATATTATGCCGATTCA GTGAAAGGCAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTAATCTGTGTGAGACATGGAACCTCGGTAAATAGCTACG TTCTCTGGTGGCTTACTGGGGCCAAAGGACTCTGGTCACCGTCTCTCTCA
161.	VL of F12Q	artificial	aa	QTVVTQEPSTLVSPGGTVTLTCSSTGAVTSGNYPNWWVQKPGQAPRGLIGGTFKFLAPGTPARFS GSLLGKKAALTLSGVQPEDEAEYCVLWYSNRWVFGGGLTVL
162.	VL of F12Q	artificial	nt	CAGACTGTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCACTTG TGGCTCCCTCGACTGGGCTGTACATCTGGCAACTACCCAACTGGTCCCAACAAAAACCAAGGTC AGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTCTCTGCCCGGTACTCTGCCAGATTCTCA GGTCCCTGCTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACGCCAGAGGATGAGGCAGA ATAATTACTGTGTCTTATGGTACAGCAACCGCTGGGTGGTGGAGGAACCAAACTGACTGTCC TA
163.	VH-P of F12Q	artificial	aa	EVQLLESGLVQPGGSLKLSCAASGFTFNSYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYVSWWAYWGQGLTVTVSS
164.	VH-P of F12Q	artificial	nt	GAGGTGCAGCTGCTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCACTTGAACCTCTCATG TGACGCCCTCTGGATTCACTTCAATAGTACGCCATGAACCTGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAAAGAAATAATAATAATAATATGCAACATATTATGCCGATTCA GTGAAAGGCAGGTTACCATCTCCAGAGATGATCAAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACCTCGGTAAATAGCTACG

165.	VL-P of F12Q	artificial	aa	TTTCCTGGTGGGCTTACTGGGGCCCAAGGACTCTGGTCACCGTCTCCTCA ELVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKPGQAPRGLIGGTKFLAPGTPARES GSLGGKAAALTLSGVQPEDEAEYYCVLWYSNRWVFGGTKLTVL
166.	VL-P of F12Q	artificial	nt	GAGTCGTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGACAGTCACACTCACTTG TGGTCCTCGACTGGGCTGTACATCTGGCAACTACCCAACTGGTCCAAACAAACAGGTC AGGCACCCCGTGGTCTAATAGGTGGACTAAGTCTCTGCCCGGCTACTCTGCCAGATTCTCA GGTCCCTGCTTGGAGGCAAGCTGCCCTCACCTCTCAGGGGTACAGCAGAGGATGAGGCAGA ATATTACTGTGTTCTATGGTACAGCAACCGCTGGGTGTTCTGGTGGAGGAACCAACTGACTGTCC TA
167.	VH-VL of F12Q	artificial	aa	EVQLVESGGGLVQPGGSLKLSAASGFTFNSYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFNGSYVSWWAYWGQGLTVTVSSGGGGS GGGSGGGGSGTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKPGQAPRGLIGGTKF LAPGTPARFSGSLLGKAAALTLGVQPEDEAEYYCVLWYSNRWVFGGTKLTVL
168.	VH-VL of F12Q	artificial	nt	CAGGTGAGCTGGTCTGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGAGCCTCTGGATTCACTTCAATAGCTACCCATGAACCTGGTCCGCCAGGTCAGGAAAGG GTTTGAATGGTGTGCTCGCATAGAAGTAAATATAATATGCAACATATTATGCCGATTCA GTGAAAGCAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTGTGTGAGACATGGAACTTCGGTAATAGCTACG TTTCTGTGGCTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCAGTGGTGGTGGTCTCT GGCGGGCGGCTCCGTGCTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCTGCACT ATCACTGGTGAACAGTCACACTCACTGTGGCTCCTCGACTGGGCTGTACATCTGGCAACT ACCAAACTGGTCCAAACAAACAGGTCAGGCACCCCGTGGTCTAATAGTGGGACTAAGTTC CTCGCCCGGTAATCTCTGCCAGATTCTCAGGCTCCCTGCTTGGAGGCAAGGCTGCCCTCACCT CTCAGGGGTACAGCAGAGATGAGGCAGAAATATTACTGTGTTCTATGGTACAGCAACCGCTGGG TGTTCCGTGGAGCAACCAACTGACTGTCTTA
169.	VH-VL-P of F12Q	artificial	aa	EVQLLESGGGLVQPGGSLKLSAASGFTFNSYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKGRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFNGSYVSWWAYWGQGLTVTVSSGGGGS GGGSGGGGSELVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKPGQAPRGLIGGTKF LAPGTPARFSGSLLGKAAALTLGVQPEDEAEYYCVLWYSNRWVFGGTKLTVL
170.	VH-VL-P of F12Q	artificial	nt	GAGTGAGCTGCTCGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGACCTCTGGATTCACTTCAATAGCTACCCATGAACCTGGTCCGCCAGGTCAGGAAAGG GTTTGAATGGTGTGCTCGCATAGAAGTAAATATAATATGCAACATATTATGCCGATTCA GTGAAAGCAGGTTACCATCTCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAA CTTGAAAACTGAGGACACTGCCGTGTACTGTGTGAGACATGGAACTTCGGTAATAGCTACG TTTCTGTGGCTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCAGTGGTGGTGGTCTCT GGCGGGCGGCTCCGTGCTGGTCTGAGCTGTTGTGACTCAGGAACCTTCACTCACCTGCACT ATCACCTGGTGAACAGTCACACTCACTGTGGCTCCTCGACTGGGCTGTACATCTGGCAACT

						ACCCAAACTGGGTCCAAACAAAAACCAGGTCAGGCACCCCGTGGTCTAATAGGTGGACTAAGTTCCTCGCCCCGGTACTCTCGCCAGATTCTCAGGCTCCCTGCTGGAGGCAAGGCTGCCCTCACCCCTCAGGGGTACAGCCAGAGATGAGGCAGAAATATTACTGTCTTATGGTACAGCAACCGCTGGGTGTTCCGTGGAGGAAACCAACTGACTGTCTTA
171.	CDR-L1 of I2C	artificial	aa			GSSTGAVTSGNYPN
172.	CDR-L2 of I2C	artificial	aa			GTKFLAP
173.	CDR-L3 of I2C	artificial	aa			VLWYSNRWV
174.	CDR-H1 of I2C	artificial	aa			KYAMN
175.	CDR-H2 of I2C	artificial	aa			RIRSKYNNYATYYADSVKD
176.	CDR-H3 of I2C	artificial	aa			HGNFGNSYISYWAY
177.	VH of I2C	artificial	aa			EVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYISYWAYWGQGLTVTVSS
178.	VH of I2C	artificial	nt			GAGGTGCAGCTGGTCGAGTCTCGAGGAGGATTGGTGACGCTGGAGGGTCATTGAAACTCTCATGTGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAATGGGTCCGCGCAGGCTCCAGGAAAGG GTTTGGAATGGGTGCTCGCATAAGAAGTAAATATAATAATATATGCAACATATTATGCCGATTCA GTGAAAGACAGGTTCAACCATCTCCAGAGATGATTCAAAATAACACATGCTCTATACAAATGAACAA CTTGAAAACAGTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACA TATCCTACTGGGCTTACTGGGGCCAAGGACTCTGGTCACCGTCTCCTCA
179.	VL of I2C	artificial	aa			QTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKFPQAPRGLIGGTKFLAPGTPARFS GSLGGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
180.	VL of I2C	artificial	nt			CAGACTGTTGTGACTCAGGAACCTTCACCTACCCGTATCATCTGGTGGGAAACAGTCACACTCACTTG TGGCTCCTCGACTGGGGCTGTTACATCTGGCAACTACCCAAACTGGGTCCAAACAAAACACAGGTC AGGCAACCCCGTGGTCTAATAAGTGGGACTAAGTTCTCGCCCCCGTACTCTCTGCCAGATTCTCA GGCTCCCTGCTTGGAAGCAAGGTCGCCCTCACCCCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGTTCTATGGTACAGCAACCGCTGGGTGTCGGTGGAGGAACCAACTGACTGTCC TA
181.	VH-P of I2C	artificial	aa			EVQLLESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGFNSYISYWAYWGQGLTVTVSS
182.	VH-P of I2C	artificial	nt			GAGGTGCAGCTGCTCGAGTCTCGAGGAGGATTGGTGACGCTGGAGGGTCATTGAAACTCTCATGTGCAGCCTCTGGATTCACTTCAATAAGTACGCCATGAATGGGTCCGCGCAGGCTCCAGGAAAGG GTTTGGAATGGGTGCTCGCATAAGAAGTAAATATAATAATATATGCAACATATTATGCCGATTCA GTGAAAGACAGGTTCAACCATCTCCAGAGATGATTCAAAATAACACATGCTCTATACAAATGAACAA CTTGAAAACAGTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACA TATCCTACTGGGCTTACTGGGGCCAAGGACTCTGGTCACCGTCTCCTCA
183.	VL-P of I2C	artificial	aa			ELVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKFPQAPRGLIGGTKFLAPGTPARFS GSLGGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL

184.	VL-P of I2C	artificial	nt	GAGTCGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGAAACAGTCACACTCATTTG TGGTCCTCGACTGGGGCTGTACATCTGGCAACTACCCAACTGGTCCACAAAACACAGGTC AGGCACCCCGTGTCTAATAGCTGGACTAAGTTCTCGCCCCCGTACTCTGCCAGATTCTCA GGTCCCTGCTTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGCAGA ATATTACTGTGTCTATGGTACAGCAACCGCTGGGTGTTCTGGTGGAGGAACCAAACTGACTGTCC TA
185.	VH-VL of I2C	artificial	aa	EVQLVESGGGLVQPGGSLKLSKAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYIQMNNIKETDVAYYCVRHGNGNSYISYWAYWQGTLVTVSSGGGGS GGGSGGGGSGTVVTQEPSTLVSPGGTVTLTCSSTGAVTSGNYPNWVQKPGQAPRGLIGGTFK LAPGTPARFSGSLIGGKAALTLGSGVQPEDEAEYYCVLWYSNRWVFGGGLKLTIVL
186.	VH-VL of I2C	artificial	nt	GAGGTGAGCTGCTGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGAGCCTCTGGATTCACTTCAATAAGTACGCCATGAATGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAAGAAGTAAATATAATAATATGCAACATATTATGCCGATTCA GTGAAAGACAGGTTACCATCTCCAGAGATGATTCAAAAACAACCTGCCTATCTACAAATGAACAA CTTGAAAACGTAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGTAATAGCTACA TATCCTACTGGCTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCAGGTGGTGGTGTCT GGGGGGGGCTCCGGTGGTGGTCTCAGACTGTGTGACTCAGGAACCTTCACTCACCGT ATCACCTGGTGAACAGTCACACTCACTGTGGTCTCTGACTGGGCTGTACATCTGGCAACT ACCCAAACTGGTCCAAACAAAACAGGTGAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTC CTGCCCGGCTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGTCGCCCTCACCT CTCAGGGGTACAGCCAGAGGATGAGGCAGAAATATTACTGTGTTCTATGGTACAGCAACCGCTGGG TGTTGGTGGAGGAACCAAACTGACTGTCTTA
187.	VH-VL-P of I2C	artificial	aa	EVQLLESGGGLVQPGGSLKLSKAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYIQMNNIKETDVAYYCVRHGNGNSYISYWAYWQGTLVTVSSGGGGS GGGSGGGGSGELVVTQEPSTLVSPGGTVTLTCSSTGAVTSGNYPNWVQKPGQAPRGLIGGTFK LAPGTPARFSGSLIGGKAALTLGSGVQPEDEAEYYCVLWYSNRWVFGGGLKLTIVL
188.	VH-VL-P of I2C	artificial	nt	GAGGTGAGCTGCTGAGTCTGGAGGAGGATTGGTGCAGCCTGGAGGGTCATTGAAACTCTCATG TGAGCCTCTGGATTCACTTCAATAAGTACGCCATGAATGGTCCGCCAGGCTCCAGGAAAGG GTTTGAATGGGTGCTCGCATAAGAAGTAAATAATAATAATATGCAACATATTATGCCGATTCA GTGAAAGACAGGTTACCATCTCCAGAGATGATTCAAAACAACCTGCCTATCTACAAATGAACAA CTTGAAAACGTAGGACACTGCCGTGTACTACTGTGTGAGACATGGAACTTCGTAATAGCTACA TATCCTACTGGCTTACTGGGGCCAAGGACTCTGGTCAACCTCTCCTCAGGTGGTGGTGTCT GGGGGGGGCTCCGGTGGTGGTCTGAGCTCGTTGTGACTCAGGAACCTTCACTCACCGT ATCACCTGGTGAACAGTCACACTCACTGTGGTCTCTGACTGGGCTGTACATCTGGCAACT ACCCAAACTGGTCCAAACAAAACAGGTGAGGCACCCCGTGGTCTAATAGGTGGGACTAAGTTC CTGCCCGGCTACTCTGCCAGATTCTCAGGCTCCCTGCTTGAGGCAAGGTCGCCCTCACCT CTCAGGGGTACAGCCAGAGGATGAGGCAGAAATATTACTGTGTTCTATGGTACAGCAACCGCTGGG

189.	EpCAM-D1-hNG2	artificial	nt	TGTTGGTGGAGGAACCAAACTGACTGTCTCTA ATGGATGAGCTGTATCATCTCTCTTGGTAGCAACAGCTACAGGTGTACACTCCACGGCGAC TTTTGCCGAGCTCAGGAAGAAATGTGTCTGTGAAAACTACAAGCTGGCCGTAAACTGCTTTGTGA ATAATAATCGTCAATGCCAGTGTACTTCAGTTGGTGCAAAAATACTGTCTATTGCTCAAAGCTG GCTGCCAAAATGTTGGTGATGAAGGCAGAAATGAATGGTCAAAACTTGGAGAAAGAGCAAAACC TGAAGGGCCCTCCAGAAACAAATGATGGGCTTTATGATCCTGACTGCGATGAGAGGGGCTCTTTA AGCCAAAGCAGTGCAACGGCACCTCCACGTGCTGGTGTGAACACTGCTGGGTCAAGAACA GACAGGACACTGAAATAAACCTGCTCTGAGCGAGTGAGAACCTACTGGATCATCTTGAACATAAA ACAAAAAGCAAGAAAAACCTTATGATAGTAAAGTTTGGGACTGCATTCAGAAAGGAGATCA CAACGGCTTATCAACTGGATCCAAAATTTATCAGAGTATTTGTGATGAGATAATGTATTCACT ATTGATCTGGTTCAAAATTTCTCAAAAAACTCAGAAATGATGTGACATAGCTGATGTGGCTTA TTATTTTGAANAAGATGTTAAGGTGAATCCTTGTTCATTTCTAAGAAATGGACCTGACAGTAA ATGGGAACAACTGGATCTGGATCCTGGTCAAACTTTAATTTATTTATGATGAAAAAGCACCT GAATTTCAATGCAGGGTCTAAAAGGCTACACTCCGACTACAAAGACGATGACGACAACTCCGT ACATCCGCTTCTTTTCGGTGAGAACCACTGGAGGTGCTGTGGCCACGGCTCTGACCGACA TAGACCTGCAGCTGCAGTTCTCACGTCACGCTCCAGCCCGAAGCCCTCCTTCTCTCTGGCAGCAGGCCA GCTGACACCTCCTGCTGCAGCTCTACTCTGGACGCTGCAGGTGAGACTTGTCTGGGCCAGGA GGAGTGAGGCTGCAGACTCCAGCAGAGACGCTGCTGAGTGACTCCATCCCCACACTGTGTGC TGACTGTCGTAGAGGGCTGGGCCACGTTGTGAGTCGATGGGTTTCTGAACGCCCTCCTCAGCAGTC CCAGAGCCCCCTAGAGGTCCCCTATGGGCTCTTTGTTGGGGCACCTGGGACCCCTTGGCCTGCC CTACCTGAGGGGAACACAGCCGACCCCTGAGGGTTGCCCTCCATGACGCCCTCAATGGCCGCA GCTCTCCGGCTCTGACCCCGATGTGCATGAGGGCTGTGCTGAAGATTTTCTGCCAGTGAT GATGTGGCCCTGGGCTTCTTGGCCCCACTCTCTGGCTGCCCTCCCTGCCCTGGGCACTCAGGA CGAAGGAACCTCGAGTTTACACTCACACACAGAGCCGGCAGGACCCCTTGGCCTTCCAGGCAG GGGCCGGCGTGGGACTTCATCTATGTGACATATTTGAGGGCCACCTGCGGGCGGTGTGGAG AAGGCCAGGTAACCGTATGTCTCACAAACAGTGTGCTGTGGCCGATGGCAGCCCCATGAGGT CAGTGTCCACATCAATGCTCACCGGCTGAAATCTCCGTGGACAGTACCCCTACGCATACTTCGA ACCGAGAGTCTCAGCTACCTGGAGCCACGGGCGAGTCTCTCTCGGGGGCTGGATGCAGAG GCTTCTGTCACCTCAGGAACACCGCTGGGCTGACACACAGAGGCCAACCAATGCCCTCCCTGCT GGGTGCTGAAAGACCTCAGTGTCAATGGCCAGAGCGGGGCTGCGGAAGCTTTGCTGACGC GCAACATGGCAGCCGGCTGCAGGCTGGAGGAGGAGATGAGGACGATGCCCTATGGCCATTAT GAAGCTTCTCCACCTGGCTCCGAGGCTTGGCCAGCCATGGAGCTGCTGAGCCATGCGTGCC TGAGCCAGGGCTGCCCTCTGTCTTGGCAATTTACCCAGTGTGACTATCAGCCCCACTAGTGG TGCCGAGGGTGGCAAGCTGGCTGAGTGGAGGAGTGTGACGGCCACGCTGGACCTGATGGAG GCTGAGCTGCGCAAAATCCAGGTGCTGTTACGGTGACCCGAGGCGCACATGGCGAGCTCGA GCTGGACATCTTGGGTGCCAGGACGAAAAATGTTCAACCTCTCTGGACGTGGTGAACCGCAAGG CCCGCTTCATCCACGATGGCTCTGAGGACACCTCCGACCACTGGTCTGGAGGTGTCTGGTGACG
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192.	EpCAM-D3-hNG2	artificial	aa	AAAACGCAACAAAGACGGGCAAGCATGACGTCAGGTCCTGACTGCCAAGCCCCGCAACGCGCCTGG CTGGTGACACCGAGACCTTTTCGCAAGGTGGAGCCAGCCAGGCATCCCGCTCACAGCTGTGCCT GGCCAGGGCCCCCTCCAGGAGCCAGCCTGACCCAGAGCTGCTGCAGTTCTGCCGGACACCCAA CCCTGCCCTTAAGAAATGCCAGTACTGGGTGTA MGWSCIIILFLVATATGVHSTATFAAAQECEVCENYKLVNCFVNNRQCQCQTSVGAQNTVICSKL AAKCLYMKAEEMNGSKLRRRAKPEGALQNNDGLYDPDCDESLFKAKQCNGTSTCWCVNATGVRRT DKDTEITCSERVITYWIIIEIKHKAREKPYDSKSLRTALQKEITTRYQLDPKFITSILYENNVIT TDI.VQNSSQKTQNDVDTADVAYYFKDVKGESL.FHSHKMDI.TVNGEQIDIDPGQTI.IYYVDFKAP EFSMQGLKVHSDYKDDDDKSRTRSGQLDGLVLFSHRGTLDGGFRFLSDGEHTSPGHFFRVIT AQKQVLLSLKGSQTLTVCPGSVQPLSSQTLRASSAGTDPQLLLYRVVRGPQLGRLFHAQQDSTG EALVNFQAQEVYAGNIIYEHMPPEPFWEAHDITLEQLSSPPAROVAATLAVAVSFEAAACQRP HLWKNKGLWVPEGQRARITVAALDASNLLASVSPSPQRSEHDVLFQVTQFPSPRGQLLVSEELHAG QPHFLQSQLAAGQIIVYAHGGGTQQDGFHFRHLQGPAGASVAGPQTSEAFATVRDVENRPPQP QASVPLRLTRGRAPISRAQLSVDPDSAPGEIEYEQRAPHNGFLSLVGGGLGPVTRFTQADVD SGRLAFVANGSSVAGIFQLSMSDASPLPMSLAVDILPSAIEVQLRAPLEVPOALGRSSLSQQQ LRVSDREEPEAAAYRLIQGPQYGHLLVGGRPVSFQFQIDQGEVVFATNSSSSHDHFRVLALA RGVNASAVNVTVRALLHVWAGGPWPQGATLRDPTVLDAGELANRTDSVPRFRLLEGPRHGRVV RVPRARTEPGGSQIVQFTQDLEDGRIGLEVGRPEGRAPGAGDSLTLELWAOQVPPAVASLDF ATEPYNAARPYSAVALLSVPEAAARTEAGKPESSTPTGEPGMASSEPAPAVAKGGFLSFLEANMFVS IIPMCLVLLALLALILPLLFIYLRKRNTKGKHDVQLVLTAKPRNGLAGDTETFRKVEPGQAIPLTAVP GGPPPGQDPPELLQFCRTPNPALKNGQYVW ATGGATGGAGCTGTATCATCTCTCTTGTGGTAGCAACAGCTACAGGTGTACATCCACGGCGAC TTTTGCCGCAGCTCAGGAAGAAATGTGTCTGTGAAAACTACAAGCTGGCCGTAAACTGCTTTGTGA ATAATAATCGTCAATGCCAGTGACTTCAGTTGGTGACAAAATACTGTCACTTTGCTCAAAGCTG GCTGCCAAAATGTTTGGTGATGAAGGCAGAAATGAATGGCTCAAACTTGGGAGAGAGCAAAACC TGAAGGGGCCCTCCAGAACAAATGATGGCTTATGATCTGACTGCGATGAGAGCGGGCTCTTTA AGGCCAAGCAGTGAACGGCACCTCCACGTGCTGGTGTGAACACTGCTGGGTGAGAAGAACAA GACAAAGCACTGAAATAACCTGCTGAGCGAGTGAGAACCTACTGGATCATCATTAACACTAAA ACACAAAGCAAGAGAAAAACCTTATGATAGTAAAGTTTGGGACTGCACTTCAGAAAGGAGATCA CAACGGTTATCAACTGGATCCAAAATTTATCAGCAGATATTTTGTATGAGAATAATGTTATCACT ATTGATCTGGTCAAAAATCTCTCAAAAACCTCAGAAATGATGTGGACATAGCTGATGTGGCTTA TTATTTTGA AAAAGATGTTAAAGGTGAATCCTTGTTCATTTCTAAGAAAATGGACCTGACAGTAA ATGGGAACAACCTGGATCTGGATCTGGTCAAACTTTAATTTATTAIGTTGATGAAAAAGCACCT GAATTTTCAATGCAGGGTCTAAAAGGGGTACACTCCGACTACAAAGACGATGACGACAAAGTCCGT ACACTCCGCTTCTTCTCGGTGAGAACCACTGGAGGTGCTGTGGCCACGGCTCTGACCGACA TAGACTGCAGCTGCAGTTCTCCAGCTCCAGCCGAGGCCCTCCTTCTCTCTGGCAGCAGGCCCA GCTGACCACTCTCTGCTGCAGCTCTACTCTGGACGCCTGCAGGTGCAGCTTGTCTCTGGCCAGGA
193.	EpCAM-D1D3-hNG2	artificial	nt	ATGGATGGAGCTGTATCATCTCTCTTGTGGTAGCAACAGCTACAGGTGTACATCCACGGCGAC TTTTGCCGCAGCTCAGGAAGAAATGTGTCTGTGAAAACTACAAGCTGGCCGTAAACTGCTTTGTGA ATAATAATCGTCAATGCCAGTGACTTCAGTTGGTGACAAAATACTGTCACTTTGCTCAAAGCTG GCTGCCAAAATGTTTGGTGATGAAGGCAGAAATGAATGGCTCAAACTTGGGAGAGAGCAAAACC TGAAGGGGCCCTCCAGAACAAATGATGGCTTATGATCTGACTGCGATGAGAGCGGGCTCTTTA AGGCCAAGCAGTGAACGGCACCTCCACGTGCTGGTGTGAACACTGCTGGGTGAGAAGAACAA GACAAAGCACTGAAATAACCTGCTGAGCGAGTGAGAACCTACTGGATCATCATTAACACTAAA ACACAAAGCAAGAGAAAAACCTTATGATAGTAAAGTTTGGGACTGCACTTCAGAAAGGAGATCA CAACGGTTATCAACTGGATCCAAAATTTATCAGCAGATATTTTGTATGAGAATAATGTTATCACT ATTGATCTGGTCAAAAATCTCTCAAAAACCTCAGAAATGATGTGGACATAGCTGATGTGGCTTA TTATTTTGA AAAAGATGTTAAAGGTGAATCCTTGTTCATTTCTAAGAAAATGGACCTGACAGTAA ATGGGAACAACCTGGATCTGGATCTGGTCAAACTTTAATTTATTAIGTTGATGAAAAAGCACCT GAATTTTCAATGCAGGGTCTAAAAGGGGTACACTCCGACTACAAAGACGATGACGACAAAGTCCGT ACACTCCGCTTCTTCTCGGTGAGAACCACTGGAGGTGCTGTGGCCACGGCTCTGACCGACA TAGACTGCAGCTGCAGTTCTCCAGCTCCAGCCGAGGCCCTCCTTCTCTCTGGCAGCAGGCCCA GCTGACCACTCTCTGCTGCAGCTCTACTCTGGACGCCTGCAGGTGCAGCTTGTCTCTGGCCAGGA

GGAGCTGAGGCTGCAGACTCCAGCAGAGACGCTGCTGAGTGACTCCATCCCCACACTGTGTGTGCTGACTGCTGAGGGCTGGGCCACAGTTGTGAGTCGATGGGTTTCTGAACGCCCTCCTCAGCAGTCCAGGAGCCCCCTAGAGGTCCCCTATGGGCTCTTTGTTGGGGCACTGGACCCCTTGGCCCTGCCCTACCTGAGGGAAACAGCCGACCCCTGAGGGTTGCCATGACGCCACCTCAATGGCCGCA			
GCCCTCCGGCCCTGACCCCCGATGTGATGAGGGCTGTGCTGAAGAGTTTTCTGCCAGTGATGTGGCCCTGGGCTTCTCTGGGCCCCACTCTCTGGCTGCCCTTCCCTGCCCTGGGCACTCAGGACGAAGAACCCCTCGAGTTTACACTACCACACAGAGCCGACGCCACCTTGGCCCTCCAGGACAGGGCCGCTGGGACTTCACTATGTGGACATATTTAGGGCCACCTGCGGGCCGTGGTGGAG			
AAGGCCAGGGTACCGTATTGCTCCACAACAGTGTGCCGTGTGGCCGATGGCAGCCCCATGAGGT			
CAGTGCCACATCAATGCTCACCGCTGAAATCTCCGTGGACCACTACCTACGCATACTTCGA			
ACCGAGGAGTCTCAGCTACCTGGAGCCACGGGCAGTCTCCTTCTCGGGGGCTGGATGCAGAG			
GCCCTCCTGTCACCTCCAGGAACACCGCTGGCCCTGACACCAGAGGCCACCAATGCCCTCCCTGCTGGCTGCATGGAAGACCTCAGTGTCAATGGCCAGAGCGGGGCTGCGGGAAGCTTGTGTGACGC			
GCAACATGGCAGCCGGCTGCAGGCTGGAGGAGGAGGATGAGGACGATGCCATATGGCCATTAT			
GAAGCTTCTCCACCTGGCTCCGAGGCTTGGCCAGCCATGGAGCTGCCCTGAGCCATGCCGTGCC			
TGAGCCAGGGCTGCCCTCTGTCTTTGCCAATTTCACCCAGCTGCTGACTATCAGCCCCACTAGTGG			
TGGCCGAGGGTGGCAGCCCTGGCTTGTGAGTGAGGCATGTGAGCCCCACGCTGACCTGATGGAG			
GCTGAGCTGCCAAATCCAGCTGCTGTTTACGCTGACCCGAGGGCACACTATGGCAGCTCGA			
GCTGGACATCCTGGGTGCCAGGCACGMAATGTTTACCCCTCCTGGACGTGGTGMACCCGAMGG			
CCCGTTCAATCCACGATGGCTCTGAGGACACCTCCGACACAGTGGTGTGGAGGTGTGGGTGACG			
GCTCGGTGCCCATGCCCTCATGCCCTTCGGAGGGCCAAACATACTCCTGCCCATCCAGGTCAA			
CCCTGTCAAATGACCCACCCACATCATCTTCCACATGGCAGCCTCATGGTGATCCTGGAACACA			
CGCAGAACCCGCTGGGGCTGAGGTTTTCCAGGCTATGACCCGGACTCTGCCCTGTGAGGGCCCTC			
ACCTTCCAGTCTTGGACCTCCTCTGGCTCCCGTGGAGCGCCGAGACCAAGCTGGGGAGCC			
GGCGACCGAGTTCTCTGCCGGAGTTGGAGCCGGCAGCCTAGTCTAIGTCCACTGGGTGGTC			
CTGCACAGGACTTGACGTTCCGGTTCAGCGATGGACTGCAGGCCAGCCCCCGGCCACGCTGAG			
GTGTTGGCCATCCGGCCGCCATACAGATCCACAGATCTCAATGGACGGCGGGCTCCTGCTGTT			
CTCACACAGAGGAACCTTGAATGGAGGCTTCCGCTTCCGCTTCTGTGACGGCGAGCACATTCCTCC			
CCGGACACTTCTCCGAGTGACGGCCAGAACAGTGTCTCCTCTCGCTGAAGGGCAGCCAGACA			
CTGACTGTCTGCCCAGGGTCCGTCCAGCCACTCAGCAGTCAGACCTCAGGGCCAGCTCCAGCCG			
AGGCACTGACCCCACTCTCTCTACGTTGCTGCTGGTGGGGCCCCCAGCTAGGCCGCTGTTC			
ACGCCCAGAGGACAGCAGGGGAGCCCTGTTGAATTCACCTCAGGCAGAGGTCTACGCTGGG			
AATATTCTGTATGAGCATGAGATGCCCCCGAGCCCTTTTGGAGGCCCATGATACCTTAGAGCT			
CCAGCTCTCTCCCGCTCCCGCCGACCTCCCGCCCACTTCTCTGCTGCTCTTCTTTGAGG			
CTGCCGTGCCCAGGCCCAAGCCACTCTGGAAGAACAAAGGTCTCTGGGTCCCCGAGGGCCAG			
CGGGCAGGATCACCGTGGTCTCTGGATGCCTCCAATCTCTTGGCCAGGCTTCCATCACCCCA			
CGGCTCAGACATGATGTGCTCTTCCAGGTACACAGTTCCCCAGCCCGGCCAGCTGTGTGTGT			

195.	EpCAM-D1D2-hNG2	artificial	nt	AELRKSOVLFSVTRGAHYGELELDILGAQARKMFTLLDVNRKARFIHDSGSDTSDQLVLEVSVT ARVPMPSCLRGGQTYLLPIQVNPVNDPPHIFPHGSLMVILEHTQKPLGPEVFAQYDPDSACEGL TFQVLGTSSGLPVERRDQPEPATEFSCRELEAGSLVYVHCGRPQADLTFRVSDGLQASPPATLK VVAIRPAIQIHRSQLDGLVLFSHRGTLDDGFRFLSDGEHTSPGHFRVTAQKQVLLSLKGSQT LTVCPGSVQPLSSQTLRASSAGTDPQLLLYRVVRGQPLGLRFLHAQDSTGEALVNFQAEVYAG NIIYHEMPPEPFWEAHDLEQLSSPPARDVAATLAVASFEEAACPQRPSHLWKNKGLWVPEGQ PARITVAALDASNLLASVPSQORSEHDVLFQVTFPSRQQLLVSEEPHAGQPHFLQSLAAGQL VYAHGGGTQQDGFHRAHLQGPAGASVAGPQTSEAFITVRDNERPPQASVPLRLTRGSRA PISRAQLSVVDPDSAPGEIEYEQRAHPNGFLSLVGGGLGPVTRFTQADVDSGRFLAFVANGSSVA GIFQLSMSDGA SPPLPMSLAVDILPSAIEVQLRAPLEVPQALGRSSLQQLRVVSDREEPEAA RLIQGPQYGHLLVGGRPSTSAFSQFQIDQGEVVFATNSSSSHDHFRVLALARGVNASAVNVTVR ALLHVWAGGPWPQGATLRLDPTVLDAGELANRTDSVPRFRLLEGRHGRVVRVPRARTEPGGSQ VEQFTQQDLEDGRIGLEVGRPEGRAPGAGDSLTLLELWQQVPPAVASLDFATEFYNAARPYSA LLSVPEAARTEAGKPESSTPTGEPGPMASSPEPAVAKGGFLSFLEANMFVIIIMCLVLLLLALI LPLLFYLRKRNKTGKHVDVQVLTAKPRNGIAGDTETFRKVEPGQAIPLTAVPGQPPPGGQDPDEL LQECRTNPALKNGQYVW
195.	EpCAM-D1D2-hNG2	artificial	nt	ATGGGATGGAGCTGTATCATCTCTTCTTGGTAGCAACAGCTACAGGTGTACACTCCACAGCGAC TTTTGCGCAGCTCAGGAAGAAATGTGTCTGTGAAAACTACAAGCTGGCCGTAAACTGCTTTGTGA ATAATAATCGTCAATGCCAGTGACTTCAAGTGGTGACAAAAATACTGTCTATTGCTCAAAAGCTG GCTGCCAAAATGTTGGTGATGAAGGCAGAAAATGAATGGCTCAAAACTTGGAGAGAGAGCAAAACC TGAAGGGCCCTCCAGAACAAATGATGGGCTTATGATCCTGACTGCGATGAGAGCGGCTCTTTA AGGCCAAGCAGTGCAACGGCACCTCCACGTGCTGGTGTGTGAACACTGCTGGGTGAGAGAACAA GACAAAGGACACTGAAATAACCTGCTCTGAGCGAGTGAGAACCTACTGGATCATCTGAAGGAGATCA ACACAAAGCAAGAGAAAAACCTTATGATAGTAAAGTTTGGGACTGCCTTCAGAAAGGAGATCA CAACGGCTTATCAACTGGATCCAAAATTTATCAGCAGTATTTGTATGAGATAATGTTATCATCT ATTGATCTGGTTCAAAATTTCTCAAAAAACTCAGAAATGATGTGACATAGCTGATGTGGCTTA TTATTTGAAAAACATGTTAAAGGTGAATCCTTGTTCATTCTAAGAAAATGGACCTGACAGTAA ATGGGAACAACCTGGATCTGGATCCTGGTCAAACTTAATTTATTTATGTTGATGAAAAGCACCT GAATTTCAATGCAGGGTCTAAAAGGGTACACTCCGACTACAAAGACGATGACGACAAATCCGT ACACTCCGCTTCTTCTCGGTGAGAACCACTGGAGGTGCTGTGGCCACGGCTCTGACCCGACA TAGACCTGACGTGCAGTTCTCCACGTCCAGCCCAAGCCCTCCTTCTCTCTGGCAGCAGGCCCA GCTGACCACTCCTGCTGCAGCTCTACTCTGACGCTGCAGGTGCAGACTTGTCTCTGGCCAGGA GGAGCTGAGGCTGCAGACTCCAGCAGAGACGCTGCTGAGTGACTCCATCCCCACACTGTGGTGC TGACTGTGTAAGGGCTGGGCCACGTTGTGAGTCGATGGGTTCTGAAACGCCCTCCTCAGCAGTC CAGAGCCCCCTAGAGTCCCCCTATGGGCTCTTGTGGGGCACTGGGACCCCTGGCCCTGCC CTACCTGAGGGGAACAGCCGACCCCTGAGGGTTGCCTCCATGCAGCCACCTCAATGGCCGCA GCCTCCTCCGGCTCTGACCCCCGATGTCATGAGGGCTGTGCTGAAGAGTTTCTGCCAGTGAT

GATGTGGCCCTGGGCTTCTCTGGGGCCCCACTCTCTGGTGCCTTCCCTGCCTGGGCACTCAGGA CGAGGAACCCCTGAGTTTACACTCACACACAGAGCCGGCAGGACCCCTTGGCCCTTCCAGGCAG GGGCGGGCGTGGGACTTCAICTATGTGACATATTTAGGGCCACCTGCGGGCCGTGTGGAG AAGGGCAGGGTACCCTATGCTCCACAAACAGTGTGCTGTGGCCGATGGCAGCCCCATGAGGT CAGTGTCCACATCAATGCTCACGGCTGGAATCTCCGTGGACCAGTACCTACGCATACTTCGA ACCGAGAGTCCCTCAGTACCTGGAGCCACGGCCCTGGCCCTGACACAGAGGCCAACAAATGCCCTCCCTGCT GGCTGCATGGAAGACCTCAGTGTCAATGGCCAGAGCGGGGCTGCGGGAAGCTTTGCTGACGC GCAATGGCAGCCGGCTGAGGCTGAGGAGGAGGATGAGGACGATGCCATATGGCCATTAT GAGCTTTCTCACCCCTGGCTCCGAGGCTTGGCCAGCCATGGAGCTGCCCTGAGCCATGCGTGCC TGAGCCAGGGCTGCCCTCTGTCTTTGCCAATTTCAACCAGCTGCTGACTATCAGCCCACTAGTGG TGGCCAGGGTGGCACAGCTGGCTTGAGTGGAGGCATGTGAGCCCACTGAGCTGAGGAGCTCGA GCTGAGCTGGCAAAATCCAGGTGCTGTTTACGCTGACCCGAGGGGCACACTATGGCGAGCTCGA GCTGACATCCTGGGTGCCCCAGGCAGAAAATGTTTCACTTCTCTGGACGTGGTGAACCGCAAGG CCGCTTCAATCCAGATGGCTCTGAGGACACCTCCGACCAGCTGCTGGAGGTGCTGGTGACG GCTCGGTGCCCATGCCCTCATGCCCTCGGAGGGCCAAACATACTCTCTGCCCATCCAGGTCAA CCCTGTCAATGACCCACCCACATCATCTTCCACATGGCAGCTCATGTGATCCTGGAACACA CGAGAAAGCCGCTGGGCTGAGGTTTCCAGGCCATGACCCGACCTGCTGCTGAGGGCCTC ACCTTCCAGGTCCTTGGCACCTCTCTGGCTCCCGTGGAGCCGAGACCACTGAGCTGGGAGCC GGCACCGAGTCTCTGCGGGAGTTGGAGGCCGCGAGCCTAGTCTATGTCCACTGCGGTGGTC CTGACAGGACTTGACGTTCCGGGTACGCGATGGAATGCACTGAGGCCAGCCCCCGGCCACGCTGAAG GTGTGGCCATCCGGCCGCCATACAGATCCACAGATCTACAGGTTGCCACTGGCCCAAGGCTC TGCCATGCCCATCTTGCCCGCCAACTGTGCTGGTGGAGACCAATGCCGTGGGCGAGGATGTGAGCG TGCTGTCCGGTCACTGGGCGCTGCACTTGGGAGCTGCAGAGCATAGTACAGTGGGTG GAGGTGCTGAGTGGTGGCCACACAGGCGTTCCACCAGCGGATGIGGAGCAGGCGCGCTGAG CTACCTGAGCACTGACCCACAGCACACGCTTACGACACCGTGGAGAACCTGGCCCTGGAGGTGC AGTGGGCGAGGATCTCTGAGCAATCTGTCTTCCAGTGACCATCCAGAGCCACTGTGTGG ATGCTGGGCTGGAGCCACTGCACACTCAGAACACCCAGCAGGAGACCTTACACAGCCCACT GGAGCCACCTGGAGAGCGAGCCCCAAGCCCCCACTTCCATTATGAGGTGGTTCAGGCTC CCAGGAAGGCAACCTTCACTACAGGCGACAAGCTGTGAGTGGCCAGGCTTCAACCAGGAT GACATACAGGCTGGCCGGTGACCTATGGGCGCACAGCTGTGCTCCTCAGAGGCGAGTGGAGCAC CTTCCGTTTCCGTGTACGGCGCCACCATATTTCTCCCACTCTATACCTTCCCATCCACATTG GTGTGACCCAGATGCGCTGTCTCACCAGTCTCTCTGCTGCTGCTGCTGAGGCTGAGGCTGAGGCT TCTCTCTCTCCTACCACTCTTGTCAACACTCTCAACACTCCAGCTACCTCTATCAGTCAAT GGAGGCGCCGCTTGGGAGGTTGGCTTGGCGTGGGACACAGGACAGCACTATGAGTACAT CCTTCAACCAATGAAGACCTGTGCTGGCGGGCTGGTCTACCAAGCATGATGACTCCGAGACCACA GAAGATGATATCCCATTTGTTGCTACCCCGCAGGGCGAGGACGATGGTGACATGGCCTGGGAGGA				
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				GGTACGGGGTGTCTTCCGAGTGGCCATCCAGCCCGTGAATGACACGCCCCCTGTGACAGCCATCA GCCGATCTTCATGTGCCCGGGTGGCGGGGCTGCTGACTACAGACGACGTGGCCCTTCAGC CATCTGACTCGGGCTTGTGACGCCACGCTGCTGCTTACCCGGAAGACCTCTCTTTGGCAG TATCGTGGCCGTAGATGAGCCACGCGGCCCATCTACCGCTTACCCAGGACCTCAGGAAGA GCGAGTACTGTTGCTGCACTCAGGGCTGACCGTGGCTGGATCCAGTGCAGGTGTCGACGGG CAACACAGGCCACTGCGTGTGAGGTGACGGCTCGGAACCTACCTCCGTGTGGCCAAACGG CTCCAGCCTTGTGGTCCCTCAAGGAGGCCAGGCCACCATCGACACGGCCGTGCTCCACCTGGACA CCAACCTCGACATCCGAGTGGGATGAGTCCACTACCAGTACAGCTGGCCCTCGCTGGGA CAACTAGTCGGGCTGTCAGCCAGCCACAGCCTTCTCCAGTACAGACTGCTGGATGGGCCGT TCTCTATAGCCACAATGGCAGCCTCAGCCCCGGAAGACACCATGGCTTCTCCGTGGAACAGGGC CAGTGCACCGATGCCACCTACAACTACAAGTGACCATTGCCCTAGAGGGCCACTGGCCCCACTGAAG CTGTCGGCACAAAGATCTACGTCTTCCAGGGAGGCGAGCTGAGATCAGAAGGGACCAAGCT GGAGGAGCCACGAGGCGAGTGCACCTGCAGACATCGTATTCTCAGTGAAGACCCACCGAGTG CCGGTACCTGCTGATGCTGCTGCGTGGCCCTTGGCAGATGAGCCACCCAGCCTGGACCTGTG CAGAGCTTCTCCAGGAGCAGTGACACAGGCAGGGTCTGTACCTGCACCTCTAGACCTGAGGC CTGAGCGATGCCCTTCTCGTGGATGTGGCCTCAGGCCCTGGTGTCTCCCTCAGGGCGTCTTG TGGAGCTGAGGTGTCGCCGCTGCCATCCACTAGAGGCGCAAACTTCAGCGTCCCTGAGGT GGCAGCCTACCTGGCCCCCTCCACTGCTCGTGTCTCCGGGCCCTACTTCCCACCTCTCCTGGG CCTCAGCCTGCAGGTGCTGGAGCCACCCACGATGGACCCCTCGAAGAGAGGACGACCTCAAG CCAGGACCTCAGCGCCTTCTCTGGAGAA"GGTGGAAGAGCAGCTGATCCGTACGTGCATGAC GGAGCGGAGACACTGACAGACAGTTTGTCTGATGGCTAATGCTCCGAGATGGATCGCCAGAG CCATCCTGTGGCCTTCACTGTCACTGTCTGCTGCTCAATGACCAACCCCCCATCTCACTACAA ACACAGGCTGCAGATGTGGAGGGGGCCACTGCGCCATCCCTCGGAGGCTCTGAGGAGCACG CACGGGACTCTGGGTCTGAGGATCTGGTCTACACCATCGAGCGACGACGACGCGGGGTAGT GCTGGGGGGCGCGGCACTGAGGTGCGAGCTTACGCGAGGCCAAATTGTTCTAGAGGCCA ACATGTTACGCTCATATCCCCATGTGCTGGTACTTCTGCTCTGCGCTCATCTGCCCCCTG CTCTTACTCTCCGAAACGCAACAAGACGGGCAAGCATGACGTCAGCTCCAGTCTGACTGCCAAGCC CCGCAACGGCCTGGCTGGTGACACCGAGACCTTTCGCAAGGTGGAGCCAGGCCAGGCCATCCCCG TCAACGCTGTGCTGGCAGGGGCCCCCTCCAGGAGGCCAGCCTGACCCAGAGCTGCTGCAGTTC TGCCGACACCCCAACCTGCCCCTTAAGAATGGCCAGTACTGGGTGTGA MGWSCTILFLVATATGVHSTATFAAAOEEVCENYKLVNCFVNNRQCQCTSVGAQNTVICSKL AAKCLVMKAEMNGSKLRRRAKPEGALQNNDGLYDPCDESLEFKAKQNGTSTCWCVNTAGVRRT DKDTEITCSERVITYWIIELKHKAREKPYDSKLSRLTAQLKEITTRYQLDPKFITSILYENNVI IDLVQNSSQKTQNDVDIADVAYYFEKDVKGESLFHSKKMDLTVNGEQLDLPQDTLIYYVDEKAP EFMSQGLKGVHSDYKDDDDKSVHSASFFGENHLEVPVATLTDIDLQLQFSTSQPEALLLLAAGP ADHLLQLYSGRLQVRLVIGQEELRLQTPAETLLSDSIPTHTVTLVVEGWATLSVDGFLNASSAV PGAPLEVYPGLFVGGTGTGLPYLRGTSRPLRGCLHAATLNGRSLRPLTPDPVHEGCAEEFSASD
196.	EpCAM-D1D2-hNG2	artificial	aa	

197.	EpCAM-hNG2	artificial	nt	DVALGFSGPHSLAAPPANGTQDEGTLEFTLTTSROAPLAFQAGGRRGDFIYVDIFEGHLRAVVE KGQGTVLLHNSVPVADGQPEHVSVINAHRLIEISVDQYPTHSTNRGVLSYLEPRGSLLLGGLDAE ASRHLQEHRLGLTPEATNASLIGCMEDLSVNGQRRGLREALLTRNMAAGCRLEEEYEDDAYGHI EAFSTLAPEAWPAMELPEPCVPEPGLPPVFANFTQLLTISPLVVAEGGTAWLEWRHVQPTLDLME AELRKSQVLFSTRGAHYGELELDILGAQARKMFTLLDVNRKARFIHDGSEDTSDQLVLESVT ARVPMFSCLRRGQTYLLPIQVNPVNDPPHII FPHGSLMVILEHTKPLGPEVFQAYDPDSACEGL TFQVLGTSSGLPVERRDQPGEPATEFSCRELEAGSLVYVHCGGPAQDLTFRVSDGLQASPPATLK VVAIRPAIQIHRSTGLRLAQGSAMPILPANLSVETNAVQDVSVLFRVTGALQFGELOKHSTGGV EGAEWATQAFHQRDVEQGRVRYLSTDPQHAYDTVENLAEVQVQEI LSNLSFPVTIQRATVW MLRLEPLHTQNTQQTTLTTHLEATLEEAGSPPTFHYEVVQAPRKNLQLQGTSLSDGQFTQD DIQAGRVTYCATARASEAVEDTFRFRVTAPPYFSPLYTFPIHIGGDPDAPVLTNVLVVPGELEG VLSADHLFVKSLSNASYLYEVMERPLRLAWRGTDKTTMTSFTNEDLLRGLVYQHDDSETT EDDIPFVATRQGESGDMAWEEVRGVFRVAIQPVNDHAPVQTI SRI FHVARGRRLLTTDDVAFS DADSGFADAQLVLRKDL LFGSIVAVDEPTRPIYRFTQEDLRKRRVLFVHSGADRGWIQLQVSDG QHQTALLEVQASEPYLRVANGSSILVPOGGQGTIDTAVLHLDNLDIRSGDEVHYHVTAGPRWG QLVRAGQPATAFSQDLDLDGAVLYSHNGSLSPEDTMFVSVEAGPVHTDATIQVTIALEGPLAPIK LVRHKIIVTFQGEAAEIRRDQLEAAQEA VPPADIVFSVKSPPSAGLYVMVSRGALADEPPSLDPV QSFSQEA VDTGRVLYLHSRPEAWSDAFSLDVASGLGAPLEGVLVELEVLPAI PLEAQNFVSPEG GSLTAPPLLRVSGPYFTLLGLSLQVLEPPQHPGLOKEDGPQARTLSAFSWRVEEQILIRYVHD GSETLTDTSFVLMANASEMDRQSHPVAFVTVLPVNDQPPILTTNTGLQMWEGATAPI PAEALRST DGDSGSEDLVYTIEQPSNGRVVLRGAPGTEVRSFTQAQLFLEANMFVI IPMCLVLLLALLLPL LFYLRKRNKTKGHDVQVLTAKPRNGLAGDTETFRKVEPGQAIPLTAVPGQPPPGQDPPELLQF CRTPNPA LKNGQYWV ATGGGATGGAGCTGTATCATCTCTCTTCTTGGTAGCAACAGCTACAGGTGTACACTCCACGGCGAC TTTTCGCCGAGCTCAGGAAGAAATGTGTCTGTGAAAACTACAAGCTGGCCGTAAACTGCTTTGTGA ATAATAATCGTCAATGCAGTGACTTCAGTTGGTGACAAAATACTGTCTATTGCTCAAAGCTG GCTGCCAAATGTTTGGTGATGAAGGCAGAAAATGAATGGCTCAAAACTTGGGAGAGAGCAAAACC TGAAGGGCCCTCCAGAACAAATGATGGGCTTTATGATCTGACTGCGATGAGAGCGGCTCTTTA AGGCCAAGCAGTGCAACGGCACCTCCACGTGCTGGTGTGTGAACACTGCTGGGTGAGAAGACA GACAGGACACTGAAATAACCTGCTGAGCGAGTGAGAACCTACTGGATCATCATTTGAACATAA ACACAAAGCAAGAGAAAAACCTTATGATAGTAAAGTTTGGGACTGCCTTCAGAAAGGAGATCA CAACGGCTTATCAACTGATCCAAAATTTATCAGGAGTATTTGATGAGATAATGTTATCACT ATTGATCTGGTTCAAAATTTCTCAAAAACTCAGAAATGATGTGGACATAGCTGATGTGGCTTA TTATTTTGA AAAAGATGTTAAAGGTGAATCCTTGTTCATTCTAAGAAAAATGGACCTGACAGTAA ATGGGAACAACACTGGATCTGGATCCTGGTCAAACTTTAATTTATTTGATGAAAAAGCACCT GAATTTCAATGCAGGGTCTAAAGGGGTACACTCCGACTACAAAGACGATGACGACAAAGTCCGT ACACTCCGCTTCTCTTTCGGTGAGAACCACTGGAGGTGCCTGTGGCCACGGCTCTGACCCGACA
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			GACATACAGGCTGGCCGGGTGACCTATGGGGCCACAGCTGTGCTCAGAGGCAGTCGAGGACAC CTTCCGTTTCCGTGTACGGGGCCACCATATTTCTCCCACTCTATACCTTCCCCATCCACATTTG GTGGTACCAGATGGCCTGTCTCACCAATGTCTCTCTGCTGGTGCCTGAGGTGGTGAGGGT GTCTCTCTGTGACCACCTCTTTGTCAAGAGTCTCAACAGTGCACAGTCCACCTATGAGGTCA GGAGCGCCCCGCTTGGGAGGTGGCTTGGCGTGGACACAGGACAAGACCACCTATGTTGACAT CCTTACCAATGAAGACCTGTTGCGTGGCCGGTGGTACACAGCATGATGACTCCGAGACCACA GAAGATGATATCCCATTTGTTGCTACCCGCCAGGGCGAGAGCATGGTGACATGGCTGGGAGGA GGTACGGGGTGTCTTCCGATGGCCATCCAGCCGTGAATGCACACACGCCCTCTGTGCAGACCATCA GCCGATCTTCCATGTGGCCCCGGGTGGGGGGCTGTGTGACTACACGACGACGTCGCCCTTCAGC GATGCTGACTCGGGCTTTGCTGACGCCACGCTGTTTACCCGAAGACCTCTCTTTTGGCAG TATCGTGGCCGTAGATGAGCCCAAGGGGCCATCTACCGCTTACCCAGGAGGACCTCAGGAAGA GGCGAGTACTGTTGCTGCACTCAGGGGTGACCGTGGCTGGATCCAGTGCAGGTGTCCGACGGG CAACACAGGCCACTGCGTGTGGAGGTGCAGGCTCGGAACCTTACCTCCGTGTGGCCAAACGG CTCCAGCCTTGTGTTCCCTAAGGAGGCCAGGGACCATCGACAGGCCGTGTCTCCACTGGACA CCAACTCGACATCCGAGTGGGATGAGGTCCACTACACGTACAGCTGGCCCTCGCTGGGGA CAACTAGTCCGGCTGGTCAGCCAGCCACAGCCTTCTCCAGACGAGACCTGTGTGATGGGCCGT TCTTATAGCCACAA TGCGAGCCTCAGCCCCGAAGACACCATGGCTTCTCCGTGGAAGCAGGGC CAGTCACACGGATGCCACCTACAAGTGACCATTTGCCCTAGAGGGCCCACTGGCCCCACTGAAG CTGTTCCGGCAXXAGXAGTCTA CTGCTTCCAGGAGAGGCGTGTGATCAGXAGGXCXAGCT GGAGGAGCCAGGAGGCAGTGCCACCTTGCAAGACATCGTATTTCTAGTGAAGAGCCACCCAGTGTG CCGGCTACCTGGTGATGGTGTCCGTGGCGCTTGGCAGATGAGCCACCCAGCCTGGACCTGTG CAGAGCTTCTCCAGGAGGCAGTGGACACAGGCAGGGTCTGTACCTGCACCTTAGACCTGAGGC CTGGAGCGATGCCTTCTCGTGGATGTGGCTCAGGCCCTGGTGTCTCCCTCGAGGGCGTCTCTG TGGAGCTGAGGTGCTGCCGCTGCCATCCCACTAGAGGGCGAAACTTCAGCGTCCCTGAGGGT GGCAGCCTCACCTGGCCCCCTCCACTGTCTCCGTGTCTCCGGGCCCTACTTCCCCACTCTCCTGGG CCTCAGCCTGCAGGTGCTGGAGCCACCCAGCATGGACCCCTCGAGAAGGAGCAGCACCTCAAG CCAGGACCTCAGCGCCTTCTCCTGGAGAAATGGTGAAGAGCAGCTGATCCGCTACGTGCATGAC GGGAGCGAGACACTGACAGACAGTTTTTGTCTGATGGCTAATGCTCCGAGATGGATCGCCAGAG CCATCCTGTGGCCTTCACTGTCACTGTCTGCTGCTCAATGACCAACCCCCCATCTCACTACAA ACACAGGCCCTGCAGATGTGGGAGGGGGCCACTGCGCCCATCTCTCGGAGGCTCTGAGGAGCACG GACGGCGACTCTGGGTCTGAGGATCTGGTCTACACCATCGAGAGCCCCAGCAACGGGCGGGTAGT GCTGCGGGGGGCGCGGGCACTGAGTGGCGGACTTACGAGGCCCAATTGACGCGGGCGGCTCG TGCTGTTTCTACACAGGAACCTGGATGGAGGTTTCCGCTTCCGCTCTCTGACGGGCGAGCAC ACTTCCCCCGACACTTCTTCCGACTGACCCCCCAGACCAACTCTCTCTCTCCCTCAACGCCAC CCAGACACTGACTGTCTGCCCCAGGTCGTCAGGCCACTCAGACCTCAGACCTCAGGGCCAGCT CCAGCGCAGGCACTGACCCCCCAGCTCCTGTCTACCGTGTGGTGGGGCCCCCAGCTAGGCCGG CTGTTCCACGCCCGCAGGACAGCACAGGGGAGGCCCTGGTGAATTTCACTCAGGCAGAGGTCTA

				CGCTGGGAAATATTCTGTATGAGCATGAGATGCCCCCGAGCCCTTTTGGGAGGCCCATGATACCC TAGAGTCCAGCTGTCTCGCCGCTGCCCGGACGTGGCCGCCACCCCTTGCTGTGGTGTGTCT TTTGAGGTGCCTGTCCCAGCGCCCCAGCCACCTCTGGAAGAACAAAGGTCTCTGGTCCCCGA GGCCAGGGCCAGGATCACCGTGGTCTCTTGATGCTCCAAATCTTTGGCCAGCGTTCCAT CACCCAGGCTCAGAGCATGATGTCTTCCAGGTACACAGTCCCAGCGCGGCCAGCTG TTGGTCCGAGGAGCCCTCCATGTGGGAGCCCACTTCTGACGCCAGTCCAGTGGTGGTCCAGG GCAGTAGTGTATGCCACGGCGGTGGGGACCCAGCAGGATGGCTTCCACTTTCTGTCGCCACC TCCAGGGCCAGCAGGGCCCTCCGTGGTGGACCCCAACCTCAGAGGCCTTTGCCATCAGGGTG AGGATGTAAATGAGCGGCCCTCAGCCACAGGCCTCTGTCCACTCCGGCTCACCCGAGGCTC TCGTGCCCCATCTCCGGGCCAGTGTGTGGTGGACCCAGACTCAGCTCCTGGGAGATTG AGTACGAGTCCAGCGGGCACCCCAACGGCTTCTCAGCCTGTTGGTGGCTGGCTGGGGCCC GTGACCCGCTCACGCAAGCCGATGTGATTCAGGGCGGTGGCTTCGTGGCCACGGGAGCAG CGTGGAGGATCTTCCAGTGTGAGCATGTCTGATGGGGCCAGCCACCCCTGCCATGTCCCTGG CTGTGACATCTACATCCGCCATCGAGTGCAGTCCGGGACCCCTGGAGTGGCCCAAGCT TTGGGGCTCTCTACTGAGCCAGCAGAGCTCCGGTGGTTTTCAGATCGGAGGAGCCAGAGGC AGCATCCGGTTGATCCAGGACCCAGTATGGGCATCTCTGGTGGCGGGGCCCACTCGG CCTCAGCCAAATCCAGATAGACCAGGGGAGTGTCTTTGCTTCACTTCACTCCTCCTCTCT CATGACCACTTCAGAGTCTGCCACTGGTAGGGTGTCAATGCATCAGCCGTAGTAAACGTAC TGTAGGGCTGTGTGATGTGGCAGTGGGCCATGGCCCAAGGTGCCACCTGCGCTGG ACCCACCGTCTAGATGTGGCAGTGGCCAAACCCAGACAGTGTGCCGCTTCCGCTC CTGAGGACCCCGCATGGCCGCTGGTCCGCTGCCCGAGCCAGGACCCGGGGGAG CCAGCTGTGGAGCATCTACTCAGCAGGACCTTGAGGACGGGAGGCTGGGCTGGAGTGGGCA GGCAGAGGGAGGGCCCCCGCCCGCAGTGACAGTCTCAGTCTGAGCTGTGGGCACAGGGC GTCCGCTGTGTGGCTCCTGGACTTTGCCACTGAGCCTTAAATGCTGCCCGGCCCTACAG CGTGGCCTGTCTAGTGTCCCGAGGCGCCCGGACGGAAGCAGGAGCCAGAGCAGCACCC CCAGGAGGAGCCAGGCCCATGGCATCCAGCCCTGAGCCGCTGTGGCCAGGAGGCTTCCTG AGCTTCTAGAGGCCAAATGTTCAGCTCATATCCCATGTGCTGTTAATTCTGTCTCTGGC GCTCATCTGCCCCCTGTCTTCTACCTCCGAAACGCAACAGACGGGCAAGCATGACGTCCAGG TCTGACTGCCAACCCCGCAAGCCCTGGTGTGACACCGAGACCTTTCGCAAGTGGAGCCA GGCAGGCCATCCCGCTCAGCTGTGCTGGCCAGGGGCCCTCCAGGAGGCCAGCCTGACCC AGAGCTGTGCAGTTCTGCCGACACCCCAACCTGCCCTTAAATGGCCAGTACTGGGTGTGA
198.	EpCAM-hNG2	artificial	aa	MGWSCIIILFLVATATGVHSTATFAAQEEECVCEYKLVNCFVNNRQCQTSVGAQNTVICSKL AAKCIYMKAFEMNGSKLGRRAKPEGALQNNDGLYDPDCDESGLFKAKQCNSTCWCVNTAGVRRT DKDTEITCSERVRYWI I IELKHKAREKPYDSKSLRTALQKEITTRYQLDPKFITSI LYENNVT I DLVQNS SQTQNDVDIADVAYYFEKDKVKGESLFHSHKMDLTVNGEQLDLDPGQTLIYYVDEKAP EFMSOGLKGVHSDYKDDDDKSVHSASFFGENHLEVPVATALTDIDLQLFSTSQPEALLLLAAGP ADHLLLQLYSGRLQRLVLGQEEILRLQTPAETLLSDSI PHTVVLTVVEGWATLSVDGFLNASSAV

			PGAPLEVPYGLFVGGTGLGLPYIRGTSRPLRGCLHAATLNGRSLRLPLTPDVHEGCAEEFSASD DVALGFSGPHSLAAFPANGTQDEGTLEFTLTQSRQAPLAFQAGRRGDFIYVDIFEGHLRAVVE KQGTVLLHNSVPVADGQPHEVSVHINAHRL EISVDQYPHTHSNRGVLSYLEPRGSLLLGGLDAE ASRHLQEHRLGLTPEATNASLLGCMEDLSVNGQRRGLREALLTRNMAAGCRLEEEYEYEDDAYGHY EAFSTLAPEAWPAMELPEPCVPEGLPPVFANFTQLLTISPLVAEGGTAWLEWRHVQPTLLDME AELRSQVLFVSVTRGAHYGELELDILGAQARKMFTLLDVNRKARFIHDGSEDTSDQLVLEVSVT ARVPMPSCLRRGQTYLLPIQVNPVNDPPHIIFPHGSLMVI LEHTKPLGPEVQAYDPDPSACEGL TFQVLGTSSGLPVERRDQGPGEPA TEFSCRELEAGSLVYVHCGGPAQDLTFRVSDGLQASPPATLK VVAIRPAIQIHRSTGLRLAQGSAMPILPANLSVETNAVGDVSVLFRVTGALQFGELOKHSTGGV EGAEWWATQAFHQRDVEQGRVRYLSTDPQHAYDTVENLALEVQVQEILSNLSFPVTIQRATVW MLRLEPLHTQNTQOETLTTAHL EATLEEAGSPPTFHYEVVQAPRKGNLQLQGTRLSDGQGTQD DIQAGRVTYGATARASEAVEDTFRFRVTAPPYFSPLYTFPIHIGGDPDAPVLTNVLVVPGE GEG VLSADHLFVKSLSNSASYLYEVMERPRGLRLAWRGTDKTTMTVTSFTNEDLLRRLVYQHDDSETT EDDIPFVATRQGESGDMAWEEVRGVFRVAIQPVNDHAPVQTI SRI FHVARGRRLLTTDDVAFS DADSGFADAQLVLRKDLLFGSIVAVDEPTRPIYRFTQEDLRKRRVL FVHSGADRGIQLVSDG QHQATALLEVQASEPYLRVANGSSLVVPGGGGTIDTAVLHLDTNLDIRSGDEVHYHVTAGPRWG QLVRAGQPATAFSQDDLLDGAVLYSHNGSLSPEDTMAFSVEAGPVHTDATLQVTIALEGPLAPLK LVRHKKIYVFQGEAAEIRRDQLEAAQEA VPPADIVFSVKSPPSAGYLVMSRGALADEPPSLDPV QSFSEQEAVDTCGRVLYLHSRPEAWSDAFSLDVASGLGAPLEGVLVELEVLPAALPLEAQNF SVPEG GSLTLAPLLRVSGPYFTLLGLSLQVLEPPQHGLPKQEDGPAQRTLSAFSWRMVEEQILIRYVHD GSETLTDSEVLMANASEMDRQSHPVAFVTVLPVNDQPPILTTNTGLQWEGATAPI PAEALRST DGDSGSEDLVYTIEQPSNGRVVLRGAPGTEVRSTQALDGGVLVFSHRGTLDGGFRFRLSDGEH TSPGHFFRVTAQKQVLLSIKGSQTLTVCPGSVQPLSSQTLRASSAGTDQPQLLLYRVVRGPQLGR LFHAQQDSTGEALVNFTQAEVYAGNILEHEMPPEPFWEAHD TLEQLSSPPARDVAATLAVVS FEAACQRP RSHLWKNKGLWVPEGQRARITVAALDASNLLASVPSPQRSEHDVLFQVTQFP SRGQL LVSEEPHAGQPHFLQSLAAGQLVYAHGGGTQDDGFHFRHLQGPAGASVAGPQTSEAFAITV RDVNERPPQPQASVPLRLTRGSRAPISRAQLSVVDPDSAPGEIEYEVQRA PHNGFTLSLVGGGLGP VTRFTQADVDSGR LAFVANGSSVAGIFQLSMSD GASPPLPMSLAVDILPSAIEVQLRAPLEVPQA LGRSSLQQQLRVVSDREEPEAA YRLIQGPQYCHLLVGGRP TSASFQFIDQGEVVF AFTNSSSS HDHFRVLALARGVNASAVNVTVRALLHVWAGGPWPQGATLRLDPTVL DAGELANRTDSVPRFRL LEGPRHGRVVRVPRARTEPGGSLVEQFTQDLEDGR LGLEVRPEGRAPGPAGDSL TLELWAQG VPPAVASLDFATEPYNAARPYSVALLSVPEAA RTEAGKPESSTPTGEPGPMASSPEPAVAKGGFL SFLEANMFSVI IPMCIVLL LALILPLLFYLRKNKTKGHDVQLTAKPRNGLAGDTETFRKVEP CQAIPLTAVPCQPPPGCQPDFELLQFCRTPNPALKNCQYVW
		artificial	nt
199.	5-10 x 12C		

				GATCGCTTCACAGGCAGTGGATCTGGAACAGATTTCACTCTCACCATCAGCAGTGTGCAGGCTGA AGCCTGGCAGTTATTACTGTGAGAAATGATTATAGTTATCCGCTCACGTTCCGTGCTGGGACCA AGCTTGAGATCAAAAGGTGTTGTTCTGGCGGGCGGCTCCSGTGGTGGTCTGAGGTG CAGCTGCTCGAGCAGTCTGGAGCTGAGCTGTAAGGCCTGGGACTTCAGTGAAGATATCCTGCAA GGCTTCTGGATACGCCTTCACTAACTACTGGTAGTTGGTAAAGCAGAGGCCTGGACATGGAC TTGAGTGGATTGGAGATATTTCCCTGGAAGTGGTAATATCAATACAAATGAGAAATTCAAGGC AAAGCCACACTGACTGCAGACAAATCTTCGAGCACAGCCTATATGCAGTCACTGACATTT TGAGGACTCTGCTGTCTATTTCTGTGCAAGACTGAGAACTGGGACAGCCTATGGACTACTGGG GCCAAGGACCAAGGTACCGTCTCTCCGAGGTGGTGGATCCGAGTGCAGCTGGTCCGATCT GGAGGAGGATTGGTGAGCCTGGAGGTCATTGAACTCTCATGTGAGCCTCTGGATTCACTT CAATAAGTACGCCATGAACGTGGTCCGCCAGGCTCCAGGAAAGGTTTGAATGGTGTCTCGCA TAAGAAATAAATAATAATATGCAACATATATCCGATTCACTGAAAGACAGTTCAACATC TCCAGAGATGATTCAAAAACACTGCCCTATCTACAAATGAACTTGAACACTGAGGACACTGC CGTACTACTGTGTGAGACATGGAACTTCGGTAATAGCTACATATCCTACTGGCTTACTGGG GCCAAGGACTCTGGTCAACGCTCTCTCAGTGGTGGTGGTCTGGCGGGCGGCTCCGGTGGT GGTGGTCTCAGACTGTTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGAACAGTCAC ACTCACTTGTGGTCTCTGACTGGGCTGTACATCTGGCAACTACCAAACTGGTCCAAACAAA AACCAGGTCAGGCACCCGCTGCTCTAATAGTGGGACTAAGTCTCTGCCCGGCTACTCTGCC AGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGGGA TGAGGCAGAAATATACTGTGTTCTATGGTACAGCAACCGCTGGGTGTTCGGTGGAGGAACCAAC TGAATGCTCTA
200.	5-10 x I2C	artificial	aa	ELVMTQSPSSLTVTAGEKVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPPLLIYWASTRESGVP DRFTGSGGTDFTLTISSVQAEADIAVYYQNDYSYPLTFGAGTKLEIKGGSGGGSGGGSEV QLLEQSGAEIVRPSTVKISCKASGYAFNWLGMVKQRPFGHLEWIGDIFPGSGNIHYNEKFKG KATLTADKSSSTAYMQLSSLTFEDSAVYFCARLRNWDEPMDYWGQGTIVTVSSGGGSEVQLVES GGGLVQPGGSLKLSCAASGFTFNKYAMNWRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDSDKNTAYLQMNLIKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTIVTVSSGGGSGGGSGG GGSTVVTQEPSTLVSPGGTIVTLTCSSTGAVTSNYPNWNVQKPGQAPRGLIGGTFKFLAPGTPA RFSGLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGTKLTIVL
201.	human PSMA construct	artificial	nt	ATGTGAAATCTCCTTCAGAAACCGACTCGGCTGTGGCCACCGCGCGCGCGCTGGCTGGTGTG CGCTGGGCGCTGCTGGCGGTGGCTTCTTCTCTCGGCTTCTCTTCTCGGTGTTTATAA AATCCTCCAATGAAGCTACTAACATTAACAAAGCATAATATGAAGCATTTTGGATGAATTG AAAGCTGAGAACATCAAGAAGTCTTATATAATTTTACACAGATACCACATTTAGCAGGAACAGA ACAAAACTTTCAGCTGCAAAAGCAAAATCAATCCAGTGGAAGAAATTTGGCCTGGATTCTGTTG AGCTAGCACATATGATGTCTCTGTTGCTACCCCAAAATAAGACTCATCCCAACTACATCTCAATA ATTAATGAAGATGGAATGAGATTTTCAACACATCATTAATTGAACCACTCTCTCCAGGATATGA AAATGTTTCGGATATGTACCACCTTTCACTGCTTTCTCTCTCAAGGAATGCCAGAGAGGGCGATC

202.	human PSMA construct	artificial	aa	TAGTGTAATGTTAACTATGCACGAACCTGAAGACTTCTTTAAATTGGAACGGGACATGAAAAATCAAT TGCTCTGGGAAAAATTGTAATTGCCAGATATGGGAAAGTTTTCAGAGGAAATAAGGTTAAAAATGC CCAGCTGCAGGGGCCAAAGGAGTCATTCTCTACTCCGACCCCTGCTGACTACTTTCCTCTCTGGGG TGAAGTCCATCCAGATGGTTGGAATCTTCTGAGGAGTGTCCAGCGTGGAATAATCCTAAAT CTGAATGGTGCAGGAGACCCCTCTCACACCAAGTTACCCAGCAAAATGAATATGCTTATAGCGGTGG AATTGCAGAGGCTGTGGTCTTCCAAGTATTCCTGTTTATCCAATGGATACTATGATGCACAGA AGCTCTAGAAAAAAATGGGTGGCTCAGCACACCACAGATAGCAGCTGGAGAGGAAGTCTCAAAGTG CCTACAAATGTTGGACCTGGCTTTACTGGAACTTTTCTACACAAAAGTCAAGATGCACATCCA CTCACCAATGAAGTGACAAGAAATTTACAATGTGATAGGTACTCTCAGAGGAGCAGTGGAAACCCAG ACAGATATGTCATTCTGGAGGTCACCGGACTCATGGGTGTTTGGTGGTATGACCCCTCAGAGT GGAGCAGCTGTGTTTATGAAATGTGAGGAGCTTTGGAACACTGAAAAAGGAAGGTGGAGACC TAGAAGAACAAATTTGTTTCAAGCTGGGATGCAGAAAGAAATTTGCTCTTCTGTTCTACTGAGT GGGCAGAGGAGAAATCAAGACTCCTTCAAGAGCGTGGCGTGGCTTATATTAATGCTGACTCATCT ATAGAAGGAAACTACACTCTGAGAGTTGATTGTACACCGCTGATSTACAGCTTGGTACACAACTT AACAAAAAGAGCTGAAAAGCCCTGATGAAGGCTTTGAAGGCAAACTCTTTATGAAAAGTTGGACTA AAAAAAGTCCCTCCCCAGAGTTCAGTGGCATGCCAGGATAAGCAAAATGGGATCTGGAAATGAT TTTGAGGTGTTCTTCCAACGACTTGAATGCTTCAAGCAGAGCAGCGGTATCTAAAAATTTGGGA AACAAAAAAATTCAGCGGCTATCCACTGTATCACAGTGTCTATGAAACATATAGTTGTTGGTGGAAA AGTTTATGATCCAAATGTTTAAATATCACTCACTGTGGCCAGGTTCCGAGGAGGATGGTGTGTTT GAGTAGCCAAATCCATAGTGTCTCTTTGATTGTTCGAGATTATGCTGATGTTTAAAGAAAGTA TGCTGACAAAAATCTACAGTATTTCTATGAAACATCCACAGGAAATGAAGACATACAGTGTATCAT TTGATTCACTTTTCTGCAGTAAAGAAATTTTACAGAAATTTGCTTCCAAAGTTCAGTGAGAGACTC CAGGACTTTGACAAAAAGCAACCCAAATAGTATTAAGAAATGATGAATGATCAACTCATGTTCTGGA AAGAGCAATTAATGATCCATTAGGTTACCAGACAGGCCCTTTTATAGGCATGTCTATGCTC CAAGCAGCCACAACAAGTATGCAGGGGAGTCATTCCCAGGAATTTAIGATGCTCTGTTTGATATT GAAAGCAAAATGGACCTTCCAAGGCTGGGAGGAAGTGAAGAGACAGATTTATGTTGCAGCCTT CACAGTGACGAGCTGCAGAGACTTTGAGTGAAGTAGCCTAA
				MNLLHETDSAVATARRPRWLCAALVLAGGFFLLGLFGWFIKSSNEATNITPKHNMKAFLDEL KAENLIKKELYNFTQIPHLAGTEQNQLAKQIQSQWKEFLGDSVELAHYDVLVLLSYPNKTHPNYISI INEDGNELENTSLFEPPPGYENVSIVPPFSAFSPQGMPEGLVYVNYARTEDFEFKLERDMKIN CSGKIVARIYGVFRGNKVNAQLAGAKGVILYSDPADYFAPGVKSYPDGWNLPGGGVQVRGNILN LINGAGDPLTPGPANEYAYRRGIAEAVGLPSIPVHPPIGYDAQKLEKMGGSAPPDSSWRGSLKV PYNVGPFTGNFSTQVKMHIHSTNEVTRIYNVIGTLRGAVEPDYVILGHRDSWVFGGIDPQS GAAVVEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRLQERGVAYINADSS IEGNYTLRVDCPTPLMYSLVHNLTKEKSPDEGFEKGSLEYSTKSPSEFSGMPRI SKLGSND FEVFFQRLGIASGRARYTKNWTNKFSGYPYLVHSYVETVELVEKFDPMFKYHLTLVAQVRGGMVF ELANSIVLFPDCRDYAVVLRKYADKIYSIMKHPQEMKTYSVSFDLSFSAVKNFTEIASKFSERL

203.	soluble fusion protein of h PSMA and m Lag3	artificial	nt	QDFDKSNPIVLRMMNDQIMFLERAFIDPLGDRPFYRHHVIYAPSSHKNKYAGESFFGIYDALFDI ESKVDPSKAWGEVKKRQIYVAFTVQAAAEITLSEVA ATGAGGAGGACCTGCTCTTGGCTTTTGTCTTCTGGGACTGCTTTGGGAAGCTCCAGTTGTGTGTC TTCAGGGCTGGAAAGAGCTCCCGTGGTGTGGCCAGGAGGAGCTCCCGTCCATCTTCCCT GCAGCTCAAAATCCCAACCTGGATCCTAACTTTCTACGAAGAGAGGGGTATCTGGCAACAT CAACAGACAGTGGCCAAACCACTCCATCCCGGCCCTTGACCTTACCAAGGGATGCCCTCGCC TAGACAAACCGCACCGGTGCTACAGGTGCTGAGCGTGGCTCCAGGAGGCTCGCGAGGGGA GGCAGCCCTGCAATCCACGTGCAGCTGGAGGAGCGGCCCTCCAGCGGGGACTTCTCTCTG TGGTTGCGCCAGCTCTGGCACCGATGGGGGAGTACCAAGCCCGTGGCTCCCGCTCCGAAACCG CGCCCTCTCTGAGTCTCCGCTGGCGTGGCCAGGCTCGATGATGCTAGTCTCTCAGGAG TCTCAAGCTGCTGATGGGTCTTTTGAACCTGCTCTTCAAGCTGCTGACCGCTCTTTTAGCTGA CTGCACTGGTCCAGGGCCAGAACCGAGTGCCTGTCTACMACTCAACCGCTCTTTTAGCTGA AACTTCTCTGTACTGCCCAAGTCAGCCCCCTGGACTCTGGACCTGGGGCTGTCTCTCACCT ACAGATGGCTTCAATGTCTCCATCAGTACAACTCAAGGTCTGGGTCTGGAGCCCGTAGCC CTTCTGACAGTACGCTGTGAAGGTCTAGGTGGAGTGCCTGTCTATTTGCCCCCAGGAGT GGGACCCCTTTCTTCTCATTGCCAAAGTGAATCTCTGGAGGAGTCTCTGAGCTCCCGCTGG CTGAAAGAGTGGCAATTTTACCCTTCACTTGAAGTGTGGTCTGGCACAGGCTGGACCTTAC ACCTGTAGCATCCATCTGCAGGACAGCAGTCAATGCCACTGTCACTTGGCGTCACTACAGT GACTCCAAATCCTTGGGTACCTGGCTCCGGGGGAGCTGTGTGAGGTAACCCCGGCAT CTGGAAGGAAAGATTTGTGTGGCTGCCCTGAACAACTGTCCAGGAGTTCCTGGGGCTGTGTG CTGGAGATTGAGGAGCCAGGCTCTTGTGTGAGCGATGGCAGTGTCACTGTACGAGGGCCAGAG GCTTCTGGAGCGACAGTACGCCGACAGTCTAGCTCAGGCGCCACAGTCTAGGAGAACTCT CAGTGACCTTAAAGGAGGCCATTCGGAGGTGGTGGATCCAAATCCTCCAATGAAGCTACTAAC ATTACTCCAAAGCATAAATATGAAGCATTTTGGATGAATGAAAGCTGAGAACATCAAGAAGTT CTTATATTAATTTTACACAGATACCAATTTAGCAGGAAACAGAAACAACTTTCAGCTTGCAAGC AAATTCATCCAGTGGAAAGAAATTTGGCTGGATTTCTGTGAGCTAGCACATTTATGATGTCTG TTGTCTACCCAAATAGACTCATCCCACTACATCTCAATAATTAATGAAGATGGAATGAGAT TTTCAACACATCATTTTGAACCACTCTCCAGGATATGAAATGTTTCGGATATTGTACCCAC CTTTCAGTGTCTTCTCTCAAGGAATGCCAGAGGGGATCTAGTGTATGTTAACTATGCACGA ACTGAGACTTCTTTAAATGGACGGGACATGAAATCAATGCTCTGGGAAATTTGTAATTCG CAGATATGGGAAAGTTTTCAGAGGAAATAAGGTAAATGCCCAGCTGGCAGGGGCCAAAGGAG TCATCTCTACTCCGACCTGTGACTACTTGTCTCTGGGTGAAGTCTATCCAGATGGTTGG AATCTCTCTGGAGGTGGTCCAGCTGGAAATATCTAAATCTGAATGTCAGGAGACCCCTCT CACACAGGTACCCAGCAAAATGAATATGCTTATAGCGTGAATTCAGAGGTGTGTGGTCTTC CAAGTATCTCTGTTTCATCCAAATGGATACATGATGCACAGAACTCTAGAAAAAATGGGTGGC TCAGACCAACAGATAGCAGTGGAGGAGGAGTCTCAAAGTCCCTACAATGTTGGACCTGGCTT TACTGGAAACTTTTCTACACAAAAAGTCAAGATGCACATCCACTCTACCAATGAAGTGAACAAGAA
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				TTTACAAATGTGATAGGTACTCTCAGAGGAGCAGTGGAACACAGACAGATATGTCTATCTCTGGGAGGT CACCGGACTCATGGGTGTTTGGTGGTATTGACCCCTCAGAGTGGAGCAGCTGTTGTTTCATGAAT TGTGAGAGCTTTGGAACACTCAAAAAGGAAGGTGGAGACCTAGAGACCAATTTGTTTGGCAA GCTGGGATGCAGAAAGAAATTTGCTCTTCTTGGTTCTACTGAGTGGCAGAGGAGAAATCAAGACTC CTTCAAGAGCGTGGCTTATATTAATGCTGACTCATCTATAGAAGGAAATACACTCTGAG AGTTGATTGTACACCCGTGATGTACAGCTTGGTACACAACTAAACAAAGAGCTGAAAAGCCCTG ATGAAGGCTTTGAAGGCAAAATCTCTTTATGAAGATTGGACTAAAAAGTCTCCCCAGAGTTC AGTGGCATGCCAGGATAAGCAAAATGGGATCTGGAATGATTTTGGGTGTTCTTCCACGACT TGGAAATGCTTCAGGCAGACACGGTATATAAAATTTGGGAAACAAACAAATTCAGCGGCTATC CACTGTATCACAGTCTATGAACATATGAGTTGGTGGAAAGTTTATGATCCAATGTTTAAA TATCACCTCACTGTGCCAGGTTCCGAGGAGGATGGTGTGTTGAGCTAGCCAATCCATAGTGT CCCTTTTGAATGTCGAGATTAIGCTGTAGTTTAAAGAAAGTATGCTGACAAAATCTACAGTATTT CTATGAACATCCACAGGAAATGAAGACATACAGTGTATCATTTGATTCACTTTTTTCTGCAGTA AAGAAATTTACAGAAATGCTTCCAAGTTCAGTGAGAGACTCCAGACTTTGACAAAAGCAACCC AATAGTATTAAAGATGATGAATGATCAACTCATGTTCTGGAAAGAGCATTTATTGATCCATTAG GGTACCAGACAGGCCTTTTATAGGCATGTCTATGCTCCAGCAGCCACAAAGTATGCA GGGAGTCATTCCAGGAATTTATGATGCTCTGTTGATATTAAGCAAGTGGACCTTCCAA GGCCTGGGAGAAAGTGAAGAGACAGATTTATGTTGAGCCTTCACAGTGCAGGAGCTGCAGAGA CTTTGAGTGAAGTAGCTCCGGGCATCATCACCATCATTGTA
204.	soluble fusion protein of h PSMA and m Lag3	artificial	aa	MREDLLGLLLGLLWEAPVSVSSPGKELPVVWAQEGAPVHLPCLSKPNLDPNLFRRGGVIWQH QPDGQPTPIPALDLHQGMPSRQAPAPGRYTVLSVAPGGLRSRQPLHPHVQLEERGLQRGDFSL WLRPALRTDAGEYHATVRLPNRALSCSLRLRVQASMIASPSGVKLSDWVLLNCFSRPRDPVS VHWFQGNRPVYNVSPRHFLAETFLILLPQVSPLDSTGWGCVLTYRDGFNVSIITYNLKVLEPVA PLTVYAAEGSRVELPCHLPPGVGTPLSLIAKWTPPGGPELVAGKSGNFTLHLEAVGLAQAGTY TCSIHLQGOQLNATVTLAVITVTPKSFGLPGSRGKLLCEVTPASGKERFVWRPLNNLSRSCPGPV LEIQEARLLAERWOCQLYEGORLLGATVYAAESSSGAHSAARRISGDLKGGHSGGSGSKSNEATN ITPKHNNKAFLELKAENIKKFLYNFTQIPHLAGTEQNFQAKQIQSQWKEFGLDSVELAHYDVL LSYPNKTHPNYISIIINEDGNEIFNTSLFEPPEPPGYENVSDIVPPSFAFPQGMPEGLVYVNYAR TEDEFFKLERDMKINC SGKIV IARYGKVFGRGNKVNAQLAGAKGVILYSDPADYFAPGVKSYDPDW NLPGGGVQRGNILNLNGAGDPLTPGYPANERYARRGIAEAVGLPSIPVHPIGYYDAQKLEKMG SAPPDSSWRGSLKVPYNVPGFTGNFSTQVKMHIHSTNEVTRIYNVIGTLRGAVEPDRYVILGG HRDSWVFGGIDPQSGAAVVEIIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRL LQERGVAYINADSSIEGNYTLRVDCITPLMYSLVHNLTKELKSPDEGFEGKSLYESWTKKSPSEF SGMPRIKSLGSGNDFEVFFQRLGIASGRARYTKNWTNKFSGYPLYSVYETIELVEKFDPMFK YHLTVAQVRGGMVFELANSIVLPFDICRDYAVVLRKYADKIYISIMKHPPQEMKTYSVSFDLSFSV KNFTEIASKFSERLQDFDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHHVYIYAPSSHNNKYA GESFPGIYDALFDIESKVDPSKAWGEVKRQIYVAAFTVQAAAEETLSEVAGSHHHHHH

205.	rat PSMA construct	artificial	nt	ATGTGAACGCTCAGCAGGACAGCGACTCCGCGGAGGCCCTGGGACGCCGCCAACGCTGGTTCTG TGCTGAACACACTGGTGTGGCTTTCACGTGAACCTTCATCATTTGGCTTCCTCTTTGGGTGGTTTA TAAACCCCTCCAATGATCTACTAGCAGTGTTCCTATCCTGGCATGAAGAAGGCATTTTGTGCAA GAATGAAGGCTGAGAACATCAAAAAATTTTATACAATTTTACACGGACACACACATTTGGCCTGGAAT GACACAAACAAATTTGAGCTTGAAAAGCAAATTCATGCCAGTGAAAAGAAATTTGGCCTGGAAT TGGTTAGTTATCTGATATGATGTCTGTGTCTTCCAAACATCATTAGCTGAACCTGCCAACATATC TCAATAATTAAATGAAGATGGAATAGATTTTCAAACATCATTAGCTGAACCTGCCAACATATC ATATGAGAAACATATCAGATGATGAGTCCACCATACAGTGCCTCTCTCCAAAGGACACCGAGG GGGATCTAGTATATGTTAACTATGCACGAACTGAAGACTTCTTAACTGGAACGGGTCAATGAAG ATCAATTGTTCTGGGAAGATTGTCTATGCCAGATATGGCAAGTGTTCAGAGGAAATAAGGTTAA AAATGCTCAGCTGGCAGGTGCAAAAGGAATCATTTCTACTCAGACCCCTGCTGATTAATCTTTGTTT CTGGGTGAAGTCCTATCCAGATGGTTGGAACCTCCCTGGAGGTGGTTTCAGCGTGAAATGTC TTAAATCTTAATGGTGCAGGTGATCCCTCACACAGGTTATCCAGCAAATGAATATGCCTACAG GCATGATTCACAGAAGCTGTGGTCTTCCAAGTATTCCTGTCCATCCAATGGATATGATGATG CACAGAACTATTAGAACATATGGTGGCTCCGACCCCTGACAGCAGCTGGAAGGAGGACTA AAAGTGCCTTACAACGTGGACCTGGCTTGTCTGGAACCTTTCAAACAAAAGGTCAAGCTGCA TATTCACCTCTACAAATAAAGTGACAAGAATCTACAATGTTATTTGGTACCCTCAAAGGAGCTGTGG AACAGACAGATATGTTATCTTGAGGGCACAGAGATGCTTGGTGTGTTGGTGGCATTGACCCCT CAGAGTGAGCAGCTGTGTTTCATGMAATGTGAGGACCTTGGMACTCTGMAAGMAAGGTTG GAGGCTTAGAAGAACTATTTTGTGTCAGCTGGGATGCTGAAGAAATTTGGCCTCTTTGGTTCTA CTAGTGGGCAGAGGAACATTCAGACTCTTACAAGAACGTGGTGTGGCTTATATCAACGCTGAT TCTTCCATAGAAGGAACTACACTCTCAGAGTTGATTCACACCACTGATGCACAGCTTAGTATA CAACCTAACAAAAGAGCTGCCAAGCCGGATGAAGGCTTTGAAGGCAATCTCTTTATGACAGCT GGAAAGAAAAAGCTCTCAACTGAGTTTCAATGGAATGCCAAGGATTAGCAAGCTGGGTCTGGC AATGATTTTGAAGTGTGTTTCCAAAGACTTGGAATGCTTCAGGCAGAGCCCGGTATACAAAAA TTGAAAAAACAAACAAAGTCAGCAGCTATCCTCTCTATCACAGTGTCTATGAAACATACGAATTGG TAGAAAAATCTATGATCCGACATTTAAATATCACCTCACTGTGGCCAGGTTGAGGAGCAATG GTATTTGAAATTGGCCAAATCTATAGTGTCTCCCTTTGACTGCCAAAGTTATGCTGTAGCTCTGAA GAAACATGCTGAGACTATCTACAACATTTCAATGAATCATCCACAGGAGATGAAGCGGTACATGA TATCATTTGATTCGCTGTTTTCTGCAGTAATAATTTTACAGATGTTGCATCCAAGTTCATCAG AGACTGCAAGACTTAGACAAGCAACCCCATATTAATGAGAAATTTGAAATGATCAGCTAATGTA TCTGGAACGTGCATTATTGATCCTTTAGGATTACCAGGAAGCCCTTCTACAGGCATATCATCT ATGCTCAAAGCAGCCACAAAGATGCAAGGAGATCATCCAGGGATTTATGATGCCCTTTT CATATTAAATAACAAAATCCATATCTTAAGCCCTCCACACAACCTCAACACACATTTCTATTGCT AGCCTTCACAGTGCAGCTGCAGCAGAGACTCTTGAGAGAAGTAGACTCCGGGGATTACAAGGACG ACGATGACAAAGTAG
206.	rat PSMA construct	artificial	aa	MNAQQDSDSAEALGRRQRWFCAAGTLVLAFTGTFTIIGFLFGWFIKPSNDSTSSVSPGMKKAFLQ

				ELKAENIKKFLYNTFTRTPHLAGTQHNFEELAKQIHAQWKEFGLDLVELSDYDVLSSYPNKTHPNYI SIINEDGNEIFKTSIAELSPPGYENISDVVPYSAFSPQGTPEGDLVYVNYARTEDFFKLERVMK INCSGKIVIARYQVFRGNKVNAQLAGAKGIILYSDPADYFVPGVKSPDGNLPGGVQRGNV LNLNGAGDPLTPGPANHEYAYRHEFTEAVGLPSIPVHPICYDDAQKLEHMGSSAPDPSWKGGGL KVPYNVGPFGAGNFSKQVKLHIHSYNKVTRIYNVIGTLKGAPEPDYVILGGHRDAWVFGGIDP QSGAAVVEHIVRTFGTLKKKGWRPRRTILFASWDAEEFGLLSTEWAEHSRLQLQERGVAYINAD SSTIEGNYTLRVDCPTLMHSLVYNLTKELPSPDEGFEKSLYDSWEKSPSTEFIGMPRI SKLSSG NDFEVFFQRLGIASGRARYTKNWNKNVSSYPLYHSVYETVELVEKFYDPTFKYHLLTVAQVRGAM VFELANSIVLPFDCCQSYAVALKKHAETIYINISMNHPOEMKAYMISFDSLFSAVNNFTDVASKFNQ RLQDLDKSNPILLRI LNDQLMYLERAFIDPLGLPRPFYRHI IYAPSSHKNKYAGESFPFIYDALF DINNKVDTSKAWREVKRQISIAAFTVQAAAE TLREVDSGDYKDDDDK
207.	mutated human PSMA antigen	artificial	nt	ATGTGGAATCTCCTGCACGAAACCGACTCGGCTGTGGCCACCGCGCGCGCGCTGGCTGTG CGCTGGGGCGCTGGTGTGGCGGGTGGCTTCTTCTCCTCGGCTTCTCCTCGGGTGGTTTATAA AATCCTCAATGAAGCTACTAACATTACTCCTCAAGCATAATATGAAAGCATTTTGGATGAATTG AAAGCTGAGAAACATCAAGAACTTCTTATATAATTTACACAGATACACATTTAGCAGGAACAGA ACAAAACTTTCAGCTTGCAAAAGCAAAATTCATCCCAGTGGAAGAATTTGGCCTGGATTTCTGTTG AGCTAGCACATTATGATGTCTGTGTCTCTACCCAAATAAGACTCATCCCACTACATCTCAATA ATTAATGAAGATGAAATGAGATTTTCAAAACATCATTAATTGAACCAACCCCTCCAGGATATGA AAAATGTTTCGGATATGTACACCTTTTCAGTGCTTCTCCTCAAGGAACACAGAGGCGGATC TAGTGTAATGTTAACTATGCACGAACTGAAGACTTCTTAAATTTGGAACGGGTTCATGAAAATCAAT TGCTCTGGGAAAAATTTGTAATTTGCCAGATATGGGAAAGTTTTCAGAGGAAATAAGTTAAAAATGC CCAGCTGGCAGGGGCCAAAGGAGTCAATCTCTACTCCGACCTGCTGACTACTTTGCTCCTGGGG TGAAGTCCATATCCAGATGGTGGAACTCTTCTCGAGGTGGTGTCCAGCGTGGAATATATCCTAAAT CTGAATGGTGCAGGAGACCTCTCACACCAAGGTTACCCAGCAATGAATATGCTTATAGCGTGG AATTGCAGAGGCTGTGGTCTTCCAAGTATTCCTGTTCATCCAATGGATACTATGATGCACAGA AGCTCCTAGAAACATATGGGTGGCTCAGCACCAAGCAGATAGCAGCTGGAGAGGAAAGTCTCAAAGTG CCCTACAAATGTTGGACCTGGCTTTGCTGGAAACTTTTCTAAACAAAAGTCAAGCTGCACATCCA CTCTACCAATGAAGTGACAAGAAATTTACAAATGTGATAGGTACTCTCAGAGGAGCATGGAAACAG ACAGATATGTCATTC TGGGAGGTCAACGGGACTCATGGGTGTTTGGTGGTATTTGACCTCAGAGT GGAGAGCTGTGTCCATGAAATTTGTAGGAGCTTTTGGAACTGAAAAAGGAGGTTGGAGACC TAGAAGAACAAATTTTGTGCAAGCTGGGATGCAGAAAGAAATTTGGTCTTCTTGGTCTCTACTGAGT GGGCAGAGGAGAAATTCAGACTCCTTCAAGAGCGTGGCGTGGCTTATATTAATGCAGACTCATCT ATAGAAAGGAAACTACACTCTGAGAGTTGATGTACACCGCTGATGTACAGCTTGGTACACAACCT AACAAAAGAGCTGAAAAGCCCTGATGAAGGCTTTGAAGGCAAAATCTCTTATGAAAGTTGGACTA AAAAAAGTCTCTCCCAAGAGTTCAAGTGGCATGCCAGGATAAGCAAAATTTGGGAAATGAT TTTGAGGTGTTCTTCCAACGACTTGGAAATTTGCTTCAGGCAGAGCACGGTATACATAAAATTTGGGA ACAAAACAAATTCAGCGGCTATCCACTGTATCACAGTGTCTATGAAACATATGAGTTGGTGGAAA

208.	mutated human PSMA antigen	artificial	aa	AGTTTATGATCCAAATGTTTAAATATACCTCACTGTGGCCCAAGTTCGAGGAGGATGGTGTGTT GAGCTAGCCAAATCCATAGTGTCCCTTTTGTATGTCGAGATTATGCTGTAGTTTAAAGAAAGTA TGCTGACAAAATCTACAGTATTTCTATGAACATCCACAGGAAATGAAGCGGTACATGGTATCAT TTGATTCACTTTTCTGCAGTAAAGAAATTTACAGAAATGCTTCCAAAGTTCAGTGAGAGACTC CAGGACTTTGACAAAAGCAACCAATAGTATTAAAGATGATGAATGATCAACTCATGTTCTGGA AAGCATTTATGATCCATTAGGGTTACAGACAGGCTTTTATAGGCATGTCTATGCTC CAAGAGCCACAAAGATATGAGGGAGTCAATCCCAGGAATTTATGATGCTGTGTTGATATT AATAACAAAGTGGACACTTCCAAGGCTGGGAGAGTGAAGACAGATTTATGTTGCAGCCCTT CACAGTGCAGCAGCTGCAGACACTTGAGTGAAGTGGCTCCGGGATTACAAGGACGACGATG ACAAGTAA MNLLHETDSAVATARRPRWLCAALVLAGFFLLGLFGWFIKSSNEATNITPKHNKAFLEDEL KAEINIKFLYNFTQIPLHLAGTEQNFQLAKQIQSQWKEFGLDSVELAHYDVLLSYPNKTHPNYISI INEDGNEIFKTSLFEPPPGYENVSIVPPFSAFSPQGTPEGLVYVNYARTEDFFKLERVMKIN CSGKI VIARYGK VFRGNKVNAQLAGAKGVILYSDPADYFAPGVKSYDPDGNLPGGVQQRGNILN LNGAGDPLTPGYPAN EYAYRRGIAEAVGLPSIPVHPIGYDQAQKLEHMGGSAPPDSSWRGSLKV PYNVGPFGA NTSKQVKLHIHSTNEVTRIYNVIGTLRGAVEPD RYVILGGHRDSWVFGGIDPQS GAAVVHEIVRSFGTLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRLQERGVAYINADSS TEGNYTLRVDC TPIMYSIVHNLTKELKSPDEGFEGKSLYESWTKKSPSEFSGMPRI SKLGSND FEVFFQRLGIASGRARYTKNWE TNKFSGYPLYHSVYETELVEKFYDPMFKYHLTV AQVRGGMVF ELANSIVLPFD CRDYAVVLRYADKIYSISMKHPQEMKAYMVSFDSLFSAVKNFTEIASKFSERL QDFDKSNPIVLRMMNDQLMFLERAFIDPLGLPDRPFYRHV IYAPSSH NKYAGESFPGIYDALFDI NNKVDTSKAWGEV KRQIYVAAFTVQAAETLSEVASGDYKDDDDK ATGTGGAACGCTCAGCAGGACAGCGACTCCGGGAGGCCCCCTGGGACGCCGCCAACGCTGGTTCGTG TGCTGGAACACATGGTGTGGCTTTCAGTGGAACCTTCATCATTTGGCTTCCTCTTTGGGTGGTTTA TAAACCCCTCCAATGATCTACTAGCAGTGTTCCTATCCTGGCATGAAGAAGGCATTTTGGCAA GAAATTGAAGGCTGAGAACATCAAAAAATTTTATACAATTTACACGGACACCCACATTTGGCAGG GACACAAACAATTTGAGCTTGCAAGCAAAATTCATGCCAGTGGAAGAAATTTGGCCTGGATT TGGTTGAGTTATCTGATTATGATGTCCTGTTGTCCTACCAAAATAGACCCATCCCAACTATATC TCAATAATTAATGAAGATGGAATGAGATTTTCAACACATCATTAGCTGAATGTCACCCCCGGG ATATGAGAAACATATCAGATGTAGTGCCACCACATACAGTGCCTTCTCTCCACAAGGATGCCAGAGG GGGATCTAGTATATGTTAACTATGCACGAACCTGAAGACTTCTTAACTGGAACGGGACATGAAG ATCAATTTGTTCTGGAAAGATTGTCATCGCCAGATATGGCAAGTGTTCAGAGGAAATAAGGTTAA AAATGCTCAGCTGGCAGGTGCAAAAGGAATCATTTCTGTACTCAGACCCCTGCTGATTACTTTGTTTC CTGGGTGAAGTCCTATCCAGATGGTTGGAACCTCCCTGGAGGTGGTGTTCAGCGTGGAAATGTC TTAAATCTTAAATGGTGCAGGTGATCCCTCACACCAAGTTATCCAGCAAAATGAATATGCCTACAG GCATGAGTTCACAGAAGCTGTCCGTCTTCCAAGTATTCCTGTCATCCATCCATTTGGATATGATGATG CACAGAAACTATTAGAAAAAATGGGTGGCTCCGCAACCCCTGACACGACGCTGGAAAGGAGGACTA
209.	mutated rat PSMA antigen	artificial	nt	

				AAAGTGCCTTACAACGTGGGACCTGGCTTTACTGAAACTTTTCAACACAAAAGGTCAAGATGCA TATTACTCTACAAATAAGTGACAAGAATCTACAATGTTATTGGTACCCTCAAAGAGCTGTGG AACACAGACAGATATGTTATCTTGGAGGGCACAGAGATGCTTGGGTGTTTGGTGGCATTGACCCCT CAGAGTGGAGCAGCTGTTGTTCAATGAAATGTCAGGACCTTGGAACTCTGAAGAAGAAAGTTG GAGGCCTAGAAAGAACTATTTTGTGTTGCAAGCTGGATGCTGAAGAATTTGGCCTTCTTGGTTCTTA CTGAGTGGCAGAGGAACATTTCAAGACTCTTACAAGAACGTGTGTGGCTTATATCAACGCTGAT TCTTCCATAGAAGGAACTACACTCTCAGAGTTGATTGCAACCACTGATGCACAGCTTAGTATA CAACCTAACAAAAGAGCTGCCAAGCCCGGATGAAGCTTTGAAGCAAACTCTCTTTATGACAGCT GGAAGAAAAAGTCTTCAACTGAGTTCAATGGAATGCCAAGGATAGCAAGCTGGGTCTGGC AATGATTTTGAAGTGTCTTCCAAAGACTTGAATGCTCAGCAGAGCCCCGGTATATAAAAA TTGAAAAACAACAAAGTCAGCAGCTATCCTCTCTATCAAGTGTCTATGAACATACGAATTGG TAGAAAAATCTATGATCCGACATTTAAATATCACCTCACTGTGCCCCAGGTTGAGGAGCAATG GTATTTGAATTGGCCAAATCTATAGTGTCTCCCTTTGACTGCCAAAGTTATGCTGTAGCTCTGAA GAAACATGCTGAGACTATCTACAACATTTCAATGAATCATCCACAGGAGATGAAGACATACAGTA TATCATTTGATTCGCTGTTTTCTGCAGTAAATAATTTTACAGATGTTGCATCCAAGTTCAATCAG AGACTGCAAGACTTAGACAAGACAACCCCATATTACTGAGAAATTTTGAATGATCAGCTAATGTA TCTGGAACGTGCATTCATTGATCCTTTAGGATTACCAGGAAGGCCCTTTCTACAGGCATATCATCT ATGCTCCAAGCAGCCACAACAGTATGCAGGAGATCATCCAGGATTTATGATGCCCTTTT GATATTGMAAGCAAGTTCGATCCTTTAAGCCCTGGAGAGAACTCAAGACACAGATTTCTATTGTC AGCCTTCACAGTGCAAGCTGCAGCAGAGACTCTGAGAGAAGTAGACTCCGGGGATTACAAGGACG ACGATGACAAGTAA
210.	mutated rat PSMA antigen	artificial	aa	MWNAQQDSDSAEALGRQRWFCAGTLVLAFTGTFTIIGLFGEFIKPSNDSTSSVSYPGMKKAFLQ ELKAENIKKFLYNFTRTPLHAGTQHNFEAKQIHAQWKEFGLDLVLELSDYDVLLSYPNKTHPNYI SIINEDGNEIFNTSLAEILSPPGYENISDVVPYSAFSPQGPPEGDLVYVNYARTEDFFKLERDMK INCSGKIVIARYGQVFRGNKVKNQAQLAGAKGIIILYSDPADYFVPGVKSPDGWNLPGGGVQVRGNV LNLNGAGDPLTPGYPANAYAYRHEFTEAVGLPSIPVHPIGYDDAQKLEKMGGSAPFDSWKGGL KVPYNVPGFTGNFSTQVKLMHIHSYNKVTRIYNVIGTLKCAVEPDYVILGCHRDAAVFFGIDP QSGAAVVHEIVRTFGTLKKKGWRPRRTILFASWDAEEFGLLGSTEWAEHSRLIQERGVAVINAD SSIEGNYTLRVDCITPLMHSLVYNLTKELPSPDEGEFEGKSLYDSWKEKSPSTEFIGMPRISKLGG NDFEVFFQRLGIAASGRARYTKNWKNNKVSSYPLYHSVEMETYSISFDSLFSAVNNFTDVASKFNQ VFELANSIVLPFDCQSYAVALKKHAETIYNI SMNHPOEMKTYISFDSLFSAVNNFTDVASKFNQ RLQDLKSNPILLRIINLNDQMLYERAFIDPLGLPGRPFVRHIIYAPSSHKNKYAGESFPFIYDALF DIESKVDPSKAWREVKRQISIAFTVQAAAEETLREVDSDGYKDDDDK (GC)AG GTG CAG CTC GAG GAG TCA GGA CCT
211.	5'-VH-family specific primer MVH1	artificial	nt	
212.	5'-VH-family specific primer MVH2	artificial	nt	

213.	5'-VH-family specific primer MVH3	artificial	nt	CAG GTC CAA CTC GAG CAG CCT GGG GCT
214.	5'-VH-family specific primer MVH4	artificial	nt	GAG GTT CAG CTC GAG CAG TCT GGG GCA
215.	5'-VH-family specific primer MVH5	artificial	nt	GA (AG) GTG AAG CTC GAG GAG TCT GGA GGA
216.	5'-VH-family specific primer MVH6	artificial	nt	GAG GTG AAG CTT CTC GAG TCT GGA GGT
217.	5'-VH-family specific primer MVH7	artificial	nt	GAA GTG AAG CTC GAG GAG TCT GGG GGA
218.	5'-VH-family specific primer MVH8	artificial	nt	GAG GTT CAG CTC GAG CAG TCT GGA GCT
219.	3'-VH primer 3'MuV/HBstEII	artificial	nt	TGA GGA GAC GGT GAC CGT GGT CCC TTG GCC CCA G
220.	5'-VK-family specific primer MUVK1	artificial	nt	CCA GTT CCG AGC TCG TTG TGA CTC AGG AAT CT
221.	5'-VK-family specific primer MUVK2	artificial	nt	CCA GTT CCG AGC TCG TGT TGA CGC AGC CGC CC
222.	5'-VK-family specific primer MUVK3	artificial	nt	CCA GTT CCG AGC TCG TGC TCA CCC AGT CTC CA
223.	5'-VK-family specific primer MUVK4	artificial	nt	CCA GTT CCG AGC TCC AGA TGA CCC AGT CTC CA
224.	5'-VK-family specific primer MUVK5	artificial	nt	CCA GAT GTG AGC TCG TGA TGA CCC AGA CTC CA
225.	5'-VK-family specific primer MUVK6	artificial	nt	CCA GAT GTG AGC TCG TCA TGA CCC AGT CTC CA
226.	5'-VK-family specific primer MUVK7	artificial	nt	CCA GTT CCG AGC TCG TGA TGA CAC AGT CTC CA
227.	3'-VK primer 3'MuV/HindIII/BsiW1	artificial	nt	TGG TGC ACT AGT CGT ACG TTT GAT CTC AAG CTT GGT CCC
228.	macaque PSMA forward primer	artificial	nt	CACTGTGGCCAGGTTGAGG
229.	macaque PSMA reverse primer	artificial	nt	GACATACCACACAAAAATTCAATACGG
230.	macaque PSMA forward primer	artificial	nt	GCTCTGCTCGCGCCGAGATGTGG

231.	macaque PSMA reverse primer	artificial	nt	ACGCTGGACACACCTCCAGG
232.	macaque PSMA forward primer	artificial	nt	GGTCTACTGAGTGGGCAGAGG
233.	macaque PSMA reverse primer	artificial	nt	ACTGTGTGTGGCTGCTTGAGC
234.	macaque PSMA forward primer	artificial	nt	GGGTGAAGTCCTATCCAGATGG
235.	macaque PSMA reverse primer	artificial	nt	GTGCTCTGCTGAAGCAATTC
236.	macaque PSMA forward primer	artificial	nt	CTCGGCTTCCTCTTCGGGTGG
237.	macaque PSMA reverse primer	artificial	nt	GCATATTCAATTTGCTGGGTAACTGG
238.	macaque PSMA	artificial	nt	ATGTGGAATCTCTGTGCACGAAACCGACTCGGCTGTGGCCACCGCGCGCCCGCTGGCTGTG CGCTGGGGCAGCTGGTGTCTGGCGGTGGCTTCTTCTCTCGGCTTCTCTTCGGATGGTTTATAA AATCCTCAGTGAAGCTACTAACATTACTCCAAAGCATAATATGAAAGCATTTTGGATGAACGTG AAAGCTGAGAAACATCAAGAAAGTTCTTACATAAATTTACACAGATACCCACATTTAGCAGGAACAGA ACAAAACCTTCAACTTGCAAAAGCAAAATCAATCCAGTGAAAGAAATTTGGCCTGGATTCTGTTG AGCTAACTCATATGATGTCTGTGTCTTACCAAAATAGACTCATCCCACTACATCTCAATCAATA ATTAAATGAAGATGGAATGAGATTTTCAACACATCATTTATTTGAACCACTCTCTGCGAGGATATGA AAATGTTTCGGATATTGTACCACTTTTCAAGCTTTCTCTCTCAAGGAATGCCAGAGGGCGATC TAGTGATGTAACTATGCACGAACCTGAAGACTTCTTAAATTTGAAACGGGACATGAAAAATCAAT TGCTCTGGGAAAAATTTAAATGCCAGATATGGGAAAGTTTTCAGAGGAAATAGGTTAAAAATGC CCAGCTGGCAGGGGCCACAGGAGTCATTCTTACTCAGACCTGCTGACTACTTTGCTCTCTGGG TAAAGTCTTATCCAGATGGTTGGAATCTTCTTGAGGTGGTGTCCAGCGTGGAATAATCCTAAAT CTGAATGGTGCAGGAGACCTCTCTCACACAGGTACCAGCAAAATGAATATGCTTATAGGCGTGG AATGGCAGAGGCTGTGGTCTTCCAAGTATCCCGTTCAATCCAATTTGGTACTATGATGCACAGA AGCTCCTAGAAAAAATGGTGGCTCAGCATCACAGATAGCAGCTGGAGAGGAAGTCTCAAAGTG CCCTACAATGTGGACCTGGCTTTACTGGAACTTTTCTACACAAAAGTCAAGATGCACATCCA CTCTACCAAGTGAAGTGACAAAGAAATTTACAATGTGATAGGTACTCTCAGAGGAGCAGTGGAAACAG ACAGATAAGTCATCTTGGAGGTCACCGGGACTCATGGGTGTTTGGTGGTATTGACCTCAGAGT GGAGCAGCTGTGTTCATGAAATGTGAGGAGCTTTGGAACGCTGAAAAAGGAGGGTGGAGACC TAGAAGAACAAATTTTGTGCAAGCTGGGATGCAGAAAGAAATTTGGTCTTCTTGGTCTCTAAT GGCAGAGGAGAAATCAAGACTCTTCAAGAGCTGGGCTGGCTTATTAATGCTGATTCGTCT ATAGAGGAAACTACACTCTGAGAGTTGATTGTACACCACTGATGTACAGCTTGGTATACAACT

239.	macaque PSMA	artificial	aa	AACAAAAGAGCTGGAAGCCCTGATGAAGGGCTTGAAGGCAAAATCTCTTTATGAAAGTTGGACTA AAAAAGTCCTTCCCCGAGTTCAGTGGCATGCCAGGATAAGCAAAATGGGATCTGGAATGAT TTTGAGGTGTTCTTCAACGACTTGAATTCCTCAGGCAGAGCAGGTATACATAAAATTTGGGA AACAAAACAAATTCAGCAGCTATCCACTGTATCACAGTGTCTATGAGACATATAGTTGGTGGAAA AGTTTATGATCCAAATGTTAAATATCACCTCACTGTGGCCAGGTTCCAGGAGGATGGTGT GAACTAGCCAAATCCGTAGTCTCCTTTTATGATGAACATCCACAGGAAATGCTGTAGTTTAAAGAAATG TGCTGACAAAATCTACAATAATTTCTATGAACATCCACAGGAAATGAAGACATACAGTGTATCAT TTGATTCATTTTCTGAGTAAAGAAATTTACAGAAATGCTTCCAGTTCCAGGAGAGACTC CGGACTTTGACAAAAGCAACCAATATTTAAGAAATGATGAATGATCAACTCATGTTTCTGGA AAGAGCAATTTATGATCCATAGGTTACAGACAGACCTTTTATAGGCATGTCATCTATGCTC CAAGCAGCCACAAAGTATGAGGGGAGTCATCCAGGAATTTATGATGCTCTGTTGATATC GAAAGCAAAATGGACCTTCCAGGCTGGGAGAAGTGAAGAGACAGATTTCTGTTGCAACCTT CACAGTCAAGCAGCTGCAGAGACTTTGAGTGAAGTGGCCTAA
240.	PSMA D3 L	artificial	aa	MNLLHETDSAVATARRPRWLCAALVLAGGFFLLGLFGWFIKSSSEATNITPKHNMKAFLDEL KAENIKKELHNFTQI PHLAGTEQNFLAKQIQSQWKEFLDVELTHYDVLVLLSYPNKTHPNYISI INEDNEIFNTSLFEPPAGYENVSIVPPFSAFSPQMPGDLVYVNYARTEDEFFKLERDMKIN CSGKIVIRYGVKVRGNKVNAQLAGATGVILYSDPADYFAPGVKSYPDGWNLPGGGVQVQGNILN LNGAGDPLTPGYPANFYAYRRGMAEAVGLPSIPVHPIGYDAQKLEKMGSSASPDSSWRGSLKV PYNVGPGFTGNFSTQVKMHIHSTSEVTRIYNVIGTLRGAVEPDRYVILGHRDSWVFGGIDPQS GAAVVHEIVRSFGLKKEGWRPRRTILFASWDAEEFGLLGSTEWAEENSRLQERGVAYINADSS IEGNYTLRVDCPTPLMYSLVNLTKELESPDEGFEGKSLYESWTKSPSEFSGMPRI SKLGSND FEVFFQRLGIASGRARYTKNWTNKFSYPLYHSVYETVELVEKFYDPMFKYHLTVAQVRGGMVF ELANSVVLPFDGRDYAVILRYADKIYINISMKHPQEMKTYSVSFDLSFAVKNFTEIASKFSERL RDFDKSNPILLRMNDQIMFLERAFIDPLGIDRPFYRHHVYIAPSSHKNYAGESFPGIYDALEFI ESKYVDPQAWGEVKKRQISVATFTVQAAAEITLSEVA
241.	PSMA D3 LCDR1	artificial	aa	DIVMTQSHKFMSTSVGDRVSIICKASQDVGTAVDWYQQKPGQSPKLLIYWASTRHTGVPDRFTGS
242.	PSMA D3 LCDR2	artificial	aa	GGTDFTLTITNVQSEDLADYFCQQYNSYPLTFGAGTMLDLK
243.	PSMA D3 LCDR3	artificial	aa	KASQDVGTAVD
244.	PSMA D3 VL	artificial	nt	WASTRHT QQVNSYPLT GACATCGTGAATGACCCAGTCCCAAGTTTCATGTCCACCTCCGTGGCGACCGGGTGTCCATCAT CTGCAAGGCCTCCAGGATGTGGCACCCCGCTGGACTGGTATCAGCAGAGAGCTGGCCAGTCCC CTAAGCTGTGATCTACTGGGCTCCACAGACACACCGCGCTGCCTGACAGATTCAACCGGCTCC GGCTCTGGCACCGACTTCACCTGACCATCACCAACGTGCAGTCCGAGGACCTGGCCGACTACTT CTGCCAGCAGTACAACTCCTACCTCTGACCTTCGGCGCTGGCACCATGCTGGACCTGAAG
245.	PSMA D3 H	artificial	aa	EVQLQQSGPELVKPTSVIRISCKTSGYTFTEYTIHWVKQSHGKSLSEWIGNINPNNGGTTYNQKFE DKATLTVDKSSSTAYMELRSLTSEDSAVYYCAAGWNFDYWGQGTTLTIVSS

246.	PSMA D3 HCDR1	artificial	aa	EYTIH	
247.	PSMA D3 HCDR2	artificial	aa	NINPNNGGTTYNQKFED	
248.	PSMA D3 HCDR3	artificial	aa	GNWEDY	
249.	PSMA D3 H	artificial	nt	GAAGTGAGCTGCAGCAGTCCGGCCCTGAGCTGGTGAAGCCTGGCACCTCCGTGCGGATCTCTTG CAAGACCTCCGGCTACACCTTACCAGAGTACACCATCCACTGGGTGAACAGTCCCAACGCAAGT CCTTGAATGGATCGGCAACATCAACCTTAACAACGGCGCACCACTACAACCAAGTTCGAG GACAAGGCAACCTTACCGTGCAGCAAGTCTCTCCACCGCTACATGGAACCTGCGGTCCCTGAC CTCCGAGGACTCCGCGTGTACTACTGCGCGCTGGTGGAACTTCGACTACTGGGGCCAGGGCA CCACTGACCGTGTCTCTC	
250.	PSMA D3 LH	artificial	aa	DIVMTQSHKFMSTSVGDRVSIICKASQDVGTAVDWYQQKPGQSPKLLIYWASTRHTGVPDRFTGS GSGTDFLTITNVQSEDLADYFCQQYNSYPLTFAGTMDLKGSGSGGGGGSEVQLQQSG PELVKPGTSVRI SCKTSGYTFEYTIHWVKQSHGKSLEWIGNINPNNGGTTYNQKFEDKATLTVD KSSSTAYMELRLSLTSEDSAVYYCAAGWNFDYWGQGTTLTVSS	
251.	PSMA D3 LH	artificial	nt	GACATCGTGATGACCCAGTCCACAAAGTTTCATGTCCACCTCCGTGGCGACCGGGTGTCCATCAT CTGCAAGGCTCCAGGATGTGGCACCGCGTGGACTGGTATCAGCAGAAAGCTGGCCAGTCCC CTAAGTGCTGATCTACTGGGCTCCACAGACACACCGCGTGCCTGACAGATTCAACGGCTCC GGCTTGGCACCGACTTCACTGACCATCAACAGTGCAGTCCGAGGACCTGGCCGACTACTT CTGCCAGCAGTACAACTCTACCTCTGACCTTCGGCGTGGCAACATGCTGGACCTGAAGGGCG GAGGGGCTCTGGCGGAGAGTGGAGGGGGGATCCGAAGTGCAGTGCAGCAGTCCGGC CCTGAGTGGTGAAGCTTGGCACCTCCGTGCGGATCTCTTGAAGACCTCCGGCTACACCTTCAC CGAGTACACCATCCACTGGTGAACAGTCCACGGCAAGTCCCTGGAATGGATGGCAACATCA ACCCTAACAAACGGCGCACCATCAACAGAAAGTTCGAGGACAAGGCCACCTGACCGTGGAC AAGTCCCTCTCCACCGCTACATGGAAGTGGGTCCCTGACCTCCGAGGACTCCGCCGTGTACTA CTGGCGCGTGGTGAAGTTCGACTACTGGGGCCAGGCCACCATGACCGTGTCTCTC	
252.	PSMA D3 LH x I2C HL	artificial	aa	DIVMTQSHKFMSTSVGDRVSIICKASQDVGTAVDWYQQKPGQSPKLLIYWASTRHTGVPDRFTGS GSGTDFLTITNVQSEDLADYFCQQYNSYPLTFAGTMDLKGSGSGGGGGSEVQLQQSG PELVKPGTSVRI SCKTSGYTFEYTIHWVKQSHGKSLEWIGNINPNNGGTTYNQKFEDKATLTVD KSSSTAYMELRLSLTSEDSAVYYCAAGWNFDYWGQGTTLTVSSGGGGSEVQLVESGGGLVQPGGSL KLSCAASGFTFNKYAMNWRQAPGKGLEWVARI RSKYNNYATYYADSVKDRFTISRDDS KNTAYL QMNNLKTEDTAVYYCVRHGNFNSYISYWAYWGQGTTLTVSSGGGGGGGGGGSGGSGTQVVTQEP SLTVSPGGTTLTCCGSSGTGAVTSGNYPNWWQKPGQAPRLIGGTFKFLAPGTPARFSGSLGGKA ALTLGQVQPEDEAEYYCVLWYSNRWVFGGKTLTVL	
253.	PSMA D3 LH x I2C HL	artificial	nt	GACATCGTGATGACCCAGTCCACAAAGTTCATGTCCACCTCCGTGGCGACCGGGTGTCCATCAT CTGCAAGGCTCCAGGATGTGGCACCGCGTGGACTGGTATCAGCAGAAAGCTGGCCAGTCCC CTAAGTGCTGATCTACTGGGCTCCACAGACACACCGCGTGCCTGACAGATTCAACGGCTCC GGCTCTGGCACCGACTTCACTGACCATCAACAGTGCAGTCCGAGGACCTGGCCGACTACTT CTGCCAGCAGTACAACTCTACCTCTGACCTTCGGCGTGGCAACATGCTGGACCTGAAGGGCG	

				GAGGGGGCTCTGGCGGCGGAGGAAGTGGAGGGGGGGATCCGAAGTGCAGCTGCAGCAGTCCGGC CCTGAGTGGTGAAGCCCTGGCACCTCCGTGGGATCTCTTGCAAGACCTCCGGGTACACCTTCAC CGAGTACACATCACTGGGTCAAAACAGTCCACGGCAAGTCCCTGGAATGGATGGCAACATCA ACCTTAACAACGGCGGCACACCTACAACCAAGATTGAGGACAAGGCCACCTGACCGTGGAC AAGTCTCTCCACCGCTACATGGAACCTGCGGTCCCTGACCTCCGAGGACTCCGCCGTGACTA CTGGCGCTGGGTGGAACCTTCGACTACTGGGCGCAGGGCAACACACTGACCGTGTCTCCGGAG GTGGTGGATCCGAGGTGACGTGGTCCAGTCTGGAGGAGGATGGTGCAGCTGGAGGGTCAATG AACTCTCATGTGACGCTCTGGATTACCTTCAATAAGTACGCCATGAACCTGGTCCGCCAGGC TCCAGGAAAGGGTTTGAATGGTTGCTCGCATAGAAGTAAATAATAATTATGCAACATAATT ATGCCGATTCAAGTGAAGACAGTTTCAACATCTCCAGAGATGATTCAAAAACACTGCTCTATCTA CAAAATGAACAACTTGAAAACTGAGGACACTGCCGTGTACTACTGTGTGAGACATGGGAACCTTCGG TAAATAGCTACATATCTACTGGCTTACTGGGCGCAAGGACTCTGGTACCGTCTCTCAGGTG GTGTGGTCTGGCGCGCGGCTCCGTGGTGGTGGTGTCTCAGACTGTGTGACTCAGGAACCT TCACTACCGTATCACTGGTGGAAACAGTCACTCACTTGTGGCTCTCGACTGGGCTTAATAGGTG ATCTGGCAACTACCAAACTGGGTCCAAACAAAACAGGTGAGGACCCCGTGGTCTAATAGGTG GGACTAAGTCTCTGGCCCCCGTACTCTGCCAGATTCTCAGGCTCCCTGCTTGGAGGCAAGGCT GCCCTACCTCTCAGGGGTACAGCCAGAGGATGAGGAGAATAATTACTGTGTCTATGTTACAG CAACCGTGGGTGTTCCGTGAGGAACCAAACTGACTGTCTTA
254.	PSMA P6 L	artificial	aa	DIVLTQSPASLAVSLGQRATISCRASESIDSYDNTFMHWYQKPGQPNNLLIFRASILESGIPAR FSGSGSGFTLTLYPVEADDVATYYCHQSIDPYTFGGGTKLEIK
255.	PSMA P6 LCDR1	artificial	aa	RASESIDSYDNTFMH
256.	PSMA P6 LCDR2	artificial	aa	RASILES
257.	PSMA P6 LCDR3	artificial	aa	HQSIDEDPYT
258.	PSMA P6 VL	artificial	nt	GACATCGTGTGACCCAGTCTCCAGCCTCCCTGGCTGTGTCTCTGGGCCAGCGGGCCACCATCTC TTGCCGGGCTCCGAGTCCATCGACTCCTACGACAACACCTTCACTGCATGGTATCAGCAGAAGC CTGGCCAGCCTCTTAACCTGCTGATCTTCGGGCTCTATCTGGAATCCGGCATCCCTGCCCGG TTCTCCGGCTCTGGCTCCGGCACCGACTTCACCTGACCATCTACCTGTGGAGGCCGACGACGT GGCCACCTACTACTGCCACCACTCCATCGAGGACCTTACACCTTCGGCGGAGGGACCAAGCTGG AAATCAAG
259.	PSMA P6 H	artificial	aa	EYVLOQSGPELVKPGASVKMSCKASGYTFTGYVMHWVKQPGQVLEWIGYINPYNDVTRYNGKFK GKATLTSDKYSSTAYMELSLTSEDSAVYYCARGENWYFDSWGRGATLTIVSS
260.	PSMA P6 HCDR1	artificial	aa	G'VMH
261.	PSMA P6 HCDR2	artificial	aa	YINPYNDVTRYNGKFKG
262.	PSMA P6 HCDR3	artificial	aa	GENWYFDS
263.	PSMA P6 H	artificial	nt	GAAGTGAGCTGCAGCAGTCCGGGCCCTGAGCTGGTGAAGCCTGGCGCCTCCGTGAAGATGTCTG CAAGGCCCTCCGGCTACACCTTCACGGCTACGTGATGCACTGGGTGAACAGAAACCCGGCCAGG

264.	PSMA P6 LH	artificial	aa	TGCTGGAATGGATCGGCTACATCAACCCCTTACAACGACGTGACCCGGTACAACGGCAAGTTCAAG GGCAAGGCCACCTGACCTCCGACAAGTACTCTCCACCGCTACATGGAACCTGTCCGGCCTGAC CTCTGAGGACTCCGCCGTGTACTGCGCAGGGCGAGAACTGGTACTACTTCGACTCCTCTGGG GCAGAGGGCTACCTTGACCGTGCTTCC
265.	PSMA P6 LH	artificial	nt	DIVLTQSPASLAVSLGQRATISCRASESIDSYDNTFMHWYQKPGQPPNLLIFRASILESGIPAR FSGSGGTDFTLTIYPVEADDDVATYYCHQSIEDPYTFGGGTGLEIKGGSGGGGGGGSEVQL QQSGPELVKPGASVKMSCKASGYTFGYVMHWKQKPGQVLEWIGYINPNVDVTRYNGKFKGKAT I.TSDKYSTAYMELISGI.TSFDSAVYYCARGENWYYFDSWGRGATITVSS GACATCGTGCTGACCCAGTCTCCAGCCTCCCTGGCTGTGTCTCTGGCCAGCGGGCCACCATCTC TTGCCGGGCTCCGAGTCCATCGACTCCTACGACAACACCTCATCGACTGGTATCAGCAGAAGC CTGCCAGCCTCCTAACCCTGCTGATCTTCGGGCTCTATCCTGGAATCCGGCATCCCTGCCCGG TTCTCCGGCTGTGGTCCGGCACCGACTTCACCCTGACCATCTACCCTGTGAGGCCGACGACGT GGCACCTACTACTGCCACAGTCCATCGAGGACCTTACACCTTCGGCGAGGACCAAGCTGG AAATCAAGGCGGAGGGGATCTGGCGGCGGAGGAAGTGGAGGGCGGATCCGAAGTGCAGCTG CAGAGTCCGGCTGAGTGGTGAAGCTGGCGCTGGCGCTCCGTGAAGATGTCTGCAAGCCTCCGG CTACACCTTCAACCGCTACGTGATGCACTGGGTGAACAGAAACCCGGCCAGGTGCTGGAATGGA TCGGCTACATCAACCCCTTACAACGACGTGACCCGGTACAACGGCAAGTTCAAGGGCAAGGCCACC CTGACCTCCGACAAGTACTCTCCACCGCTACATGGAACCTGTCCGGCCTGACCTCTGAGGACTC CGCCGTGACTACTGCGCAGGGCGGAGAACTGGTACTACTTCCGACTCTCTGGGCGAGAGCGGCTA CCTACCGCTGCTCTTCC
266.	PSMA P6 LH x I2C HL	artificial	aa	DIVLTQSPASLAVSLGQRATISCRASESIDSYDNTFMHWYQKPGQPPNLLIFRASILESGIPAR FSGSGGTDFTLTIYPVEADDDVATYYCHQSIEDPYTFGGGTGLEIKGGSGGGGGGGSEVQL QQSGPELVKPGASVKMSCKASGYTFGYVMHWKQKPGQVLEWIGYINPNVDVTRYNGKFKGKAT LTS DKYSTAYMELISGLTSED SAVYYCARGENWYYFDSWGRGATLTVSSGGGGSEVQLVESGGGL VQPGGSLKLSCAA SGFTFNKYAMNWRQAPGKLEWVARIRSKYNNYATYYADSVKDRFTISRDD SKNTAYLQMNLIKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLTVTVSSGGGGSGGGSGGGSSQ TVVTFEPSTLVSPGGTVTLTCCSSTGAVTSGNYPNWVQKPGQAPRGLIGGTFKFLAPGTPARFSG SLLGGKAALTL SGVQPEDEAEYCYVLWYSNRWVFGGGLKLTVL
267.	PSMA P6 LH x I2C HL	artificial	nt	GACATCGTGCTGACCCAGTCTCCAGCCTCCCTGGCTGTGTCTCTGGCCAGCGGGCCACCATCTC TTGCCGGGCTCCGAGTCCATCGACTCCTACGACAACACCTTCATCGACTGGTATCAGCAGAAGC CTGCCAGCCTCCTAACCTGCTGATCTTCGGGCTCTATCCTGGAATCCGGCATCCCTGCCCGG TTCTCCGGCTGTGGTCCGGCACCGACTTCACCCTGACCATCTACCCTGTGAGAGCCGACGACGT GGCACCTACTACTGCCACAGTCCATCGAGGACCTTACACCTTCGGCGAGGACCAAGCTGG AAATCAAGGCGGAGGGGATCTGGCGGCGGAGGAAGTGGAGGGCGGATCCGAAGTGCAGCTG CAGCAGTCCGGCTGAGTGGTGAAGCCTGGCGCTCCGTGAAGATGTCTGCAAGCCTCCCGG CTACACCTTCAACCGCTACGTGATGCACTGGGTGAACAGAAACCCGGCCAGGTGCTGGAATGGA TCGGCTACATCAACCCCTTACAACGACGTGACCCGGTACAACGGCAAGTTCAAGGGCAAGGCCACC

				CTGACCTCCGACAAAGTACTCTCCACCGCTACATGGAACCTGTCCGGCCTGACCTCTGAGGACTC CGCGGTACTACTGCGCCAGGGCGAGAACTGGTACTACTTCCGACTCCTGGGCGAGAGCGGCTA CCCTGACCGTGTCTTCCGAGGTGGTGATCCGAGGTGCAGCTGGTCGAGTCTGGAGGAGGATTG GTGACGCTGGAGGGTCATGTGAAACTCTCATGTGCAGCCTCTGGATTCACTTCAATAAGTACGC CATGAACCTGGTCCGCTCAGGCTCAGGAAAGGTTTGAATGGTGTCTCGCATAGAAAGTAAAT ATAATAATTATGCAACATATTATGCCGATTAGTGAAGACAGGTTACCATCTCCAGAGATGAT TCAAAAACACTGCTTATCTACAAATGAACAATTGAAAACTGAGGACA CTGCCGTGTACTACTG TGTGACACATGGGAACTTCGGTAATAGCTACATATCTACTGGGCTTACTGGGGCCAAGGGACTC TGGTACCGTCTCCTCAGTGGTGGTGGTCTGGCGGGCGGCTCCGGTGGTGGTCTCTCAG ACTGTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGAACAGTCACACTCACTTGTGG CTCCTGACTGGGCTGTATCATCTGGCAACTACCCAACTGGGTCCAAACAAAACAGGTCAAG CACCCGTTGCTAATAGTGGGACTAAGTTCTCGCCCCGGTACTCTGCCAGATTCTCAGGC TCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGGATGAGGCAGAATA TTACTGTCTCTATGTTACAGCAACCGTGGTGTTCGGTGGAGSAACCAACTGACTGTCTCTA
268.	PSMA P1 L	artificial	aa	DIQMTQSPSSLSASVGDRTVITCRASQGISNYLAWYQQKTKGVPKFLIYEASTLQSGVPSRFSGG GSGTDFTLTISLQPEDVATYYCQNYNSAPFTFGPGTKVDIK
269.	PSMA P1 LCDR1	artificial	aa	RASQGISNYLA
270.	PSMA P1 LCDR2	artificial	aa	EASTLQS
271.	PSMA P1 LCDR3	artificial	aa	QNYNSAPFT
272.	PSMA P1 VL	artificial	nt	GACATCCAGATGACCCAGTCCCCCTCCTCCCTGTCTGCCCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGGCTCCAGGGCATCTCCAACCTACCTGGCTGGTATCAGCAGAAACCCGGCAAGGTGC CCAAGTTCCTGATCTACGAGGCTCCACCTGCAGTCCGGCTGCCCTCCAGATTCTCTGGCGGC GGATCCGGCACCGACTTCACTGACCTGACCATCTCCAGCCTGCAGCCTGAGGACGTGGCCACCTACTA CTGCCAGAACTACAACTCCGCCCTTTCACCTTCGGCCCTGGCAACCAAGTGGACATCAAG
273.	PSMA P1 H	artificial	aa	QVQLVESGGGVVQPPGRSLRLSCAASGFAFSRYGMHWVRQAPGKGLEWVAVI WYDGSNKYYADSVK GRFTISRDN SKNTQY LQMNSLRAEDTAVYYCARGGDFLYYYGMDVWGQGTITVTVSS
274.	PSMA P1 HCDR1	artificial	aa	RYGMH
275.	PSMA P1 HCDR2	artificial	aa	VIWYDGSNKYYADSVK
276.	PSMA P1 HCDR3	artificial	aa	GGDFLYYYGMDV
277.	PSMA P1 H	artificial	nt	CAGTGGACGTGCTGAGTCTGGCGGAGGGTGGTCCAGCCTGGCCGGTCCCTGAGACTGTCTTG CGCCGCTCCGGCTTCGCTTCTCCAGATACGGCATGCACTGGTGGCGCAGGCTCCAGGCAAGG GACTGGAATGGTGGCCGTGATTTGGTACGACGGCTCCAACAAGTACTACGCCGACTCCGTGAAG GGCCGGTTCACCATCTCCCGGACAACTCCAAGAACACCCAGTACCTGCAGATGAACCTCCCTGAG GGCAGAGGACACCGCGTGTACTACTGCGCCAGAGGGCGGACTTCTCTGTACTACTACTATTACG GCATGGACGTGTGGGGCCAGGGCACCCCGTGACAGTGTCTTCC
278.	PSMA P1 LH	artificial	aa	DIQMTQSPSSLSASVGDRTVITCRASQGISNYLAWYQQKTKGVPKFLIYEASTLQSGVPSRFSGG

279.	PSMA P1 LH	artificial	nt	GGSTDFLTLT ISSLPEDVATYYQNYNSAPFTFGPGTKVDIKGGSGSGGGSGVQVLES GGVVQPGFSLRLSCAASGFAFSGYGMHWVRQAPGKLEWVAWIYDGSNKYYADSVKGRFTISR NSKNTQYLQMNLSLRAEDTAVYYCARGGDFLYYYGMDVWGQGTITVSS GACATCCAGATGACCCAGTCCCTCCCTCCCTGCTGCTCCGTCGGCGACAGAGTGACCATCAC CTGCCGGGCTCCAGGCATCTCAACTACCTGGCTGGTATCAGCAGAAAACCGCAAGGTGC CCAAGTTCCTGATCTACGAGGCTCCACCTGCAGTCCGGCTGCTTCCAGATTCTCTGGCGG GGATCCGGCACCGACTTCACTGACCATCTCCAGCTGCAGCTGAGGACGTGGCCACTACTA CTGCCAGAACTACAACTCCGCCCTTTCACCTTCGGCCCTGGCAACCAAGTGGACATCAAGGGG GAGGGGCA GTGGCGGAGGAGTGGAGGGGGGATCTCAGTGCAGTGGTCGAGTCTGGC GGAGGGTGGTCCAGCTGGCCGCTCCCTGAGACTGTCTTGGCCGCTCCGGCTTCGCTTCTC CAGATACGGCATGCACTGGTGGCCAGGCTCCAGGCAAGGACTGGAATGGTGGCCGCTGATT GGTACGACGGCTCCAACTAGTACGCGACTCCGTGAGGGCCGTTTCACTATCTCCCGGAC AACTCCAAGAACACCCAGTACCTGCAGATGAATCCCTGAGGGCAGAGGACACCGCTGTACTA CTGCCAGAGAGGGGCGACTTCTGTACTACTACTATTACGGCATGGACGTGTGGGGCCAGGGCA CCACCGTGACAGTGTCTTC
280.	PSMA P1 LH x I2C HL	artificial	aa	DIQMTQSPSSLSASVGDRTVITCRASQGISNYLAWYQQKTKVPKFLIYEASTLQSGVPSRFS GGSTDFLTLT ISSLPEDVATYYQNYNSAPFTFGPGTKVDIKGGSGSGGGSGVQVLES GGVVQPGFSLRLSCAASGFAFSGYGMHWVRQAPGKLEWVAWIYDGSNKYYADSVKGRFTISR NSKNTQYLQMNLSLRAEDTAVYYCARGGDFLYYYGMDVWGQGTITVSSSGGGSEVQVLES LVQPGSLKLSAASGFTFNKYAMNWRQAPGKLEWVAIRSKYNNYATYADSVKDRFTISR DSKNTAYLQMNLTEDTAVYYCVRHGNGNSYISYWAYWGQGTITVSSSGGGSGGGSGGG QTVVTQEPSSLTVSPGGTVTLTCCSSTGAVTSGNYPNWVQKPGQAPRGLIGGTFKFLAPGTPARF GSLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGKLTIVL GACATCCAGATGACCCAGTCCCTCCCTCCCTGCTGCTCCGTCGGCGACAGAGTGACCATCAC CTGCCGGGCTCCAGGCATCTCAACTACCTGGCTGGTATCAGCAGAAAACCGCAAGGTGC CCAAGTTCCTGATCTACGAGGCTCCACCTGCAGTCCGGCTGCTTCCAGATTCTCTGGCGG GGATCCGGCACCGACTTCACTGACCATCTCCAGCTGCAGCTGAGGACGTGGCCACTACTA CTGCCAGAACTACAACTCCGCCCTTTCACCTTCGGCCCTGGCAACCAAGTGGACATCAAGGGG GAGGGGCA GTGGCGGAGGAGTGGAGGGGGGATCTCAGTGCAGTGGTCGAGTCTGGC GGAGGGTGGTCCAGCTGGCCGCTCCCTGAGACTGTCTTGGCCGCTCCGGCTTCGCTTCTC CAGATACGGCATGCACTGGTGGCCAGGCTCCAGGCAAGGACTGGAATGGTGGCCGCTGATT GGTACGACGGCTCCAACTAGTACGCGACTCCGTGAGGGCCGTTTCACTATCTCCCGGAC AACTCCAAGAACACCCAGTACCTGCAGATGAATCCCTGAGGGCAGAGGACACCGCTGTACTA CTGCCAGAGAGGGGCGACTTCTGTACTACTACTATTACGGCATGGACGTGTGGGGCCAGGGCA CCACCGTGACAGTGTCTTCGGAGGTGGTGGATCCGAGGTGCAGTGGTCGAGTGTGGAGGAGGA TTGGTGCAGCCTGGAGGGTCAATGAACCTCTCATGTGACGCTCTGGATTCACTTCAATAAGTA CGCCATGAACCTGGGTCCGCCAGGCTCCAGGAAAGGTTTGGAAATGGTTCCTCGCATAGAAGTA
281.	PSMA P1 LH x I2C HL	artificial	nt	GACATCCAGATGACCCAGTCCCTCCCTCCCTGCTGCTCCGTCGGCGACAGAGTGACCATCAC CTGCCGGGCTCCAGGCATCTCAACTACCTGGCTGGTATCAGCAGAAAACCGCAAGGTGC CCAAGTTCCTGATCTACGAGGCTCCACCTGCAGTCCGGCTGCTTCCAGATTCTCTGGCGG GGATCCGGCACCGACTTCACTGACCATCTCCAGCTGCAGCTGAGGACGTGGCCACTACTA CTGCCAGAACTACAACTCCGCCCTTTCACCTTCGGCCCTGGCAACCAAGTGGACATCAAGGGG GAGGGGCA GTGGCGGAGGAGTGGAGGGGGGATCTCAGTGCAGTGGTCGAGTCTGGC GGAGGGTGGTCCAGCTGGCCGCTCCCTGAGACTGTCTTGGCCGCTCCGGCTTCGCTTCTC CAGATACGGCATGCACTGGTGGCCAGGCTCCAGGCAAGGACTGGAATGGTGGCCGCTGATT GGTACGACGGCTCCAACTAGTACGCGACTCCGTGAGGGCCGTTTCACTATCTCCCGGAC AACTCCAAGAACACCCAGTACCTGCAGATGAATCCCTGAGGGCAGAGGACACCGCTGTACTA CTGCCAGAGAGGGGCGACTTCTGTACTACTACTATTACGGCATGGACGTGTGGGGCCAGGGCA CCACCGTGACAGTGTCTTCGGAGGTGGTGGATCCGAGGTGCAGTGGTCGAGTGTGGAGGAGGA TTGGTGCAGCCTGGAGGGTCAATGAACCTCTCATGTGACGCTCTGGATTCACTTCAATAAGTA CGCCATGAACCTGGGTCCGCCAGGCTCCAGGAAAGGTTTGGAAATGGTTCCTCGCATAGAAGTA

282.	PSMA P2 L	artificial	aa	AATATAATAATTATGCAACATATTATGCCGATTTCAGTGAAGACAGGTTCCACCATCTCCAGAGAT GATTCAAAAAACACTGCCTATCTACAAATGAACAACTTGAAACTGAGGACACTGCCGTGTACTA CTGTGTGAGACATGGGAACCTCGGTAATAGTACATATCCTACTGGCTTACTGGGGCCAAAGGA CTTCTGGTCAACCGTCTCTCAGGTGGTGGTCTTCTGGCGCGCGCTCCGGTGGTGGTGGTCT CAGACTGTTGACTCAGGAACCTTCACTCACCGTATCACTTGGTGAACAGTCACACTCACTTG TGGCTCCTCAGCTGGGTGTTACATCTGGCAACTACCAAACTGGGTCCAACAAAACACAGGTC AGGCAACCCGTGGTCTAATAGTGGGACTAAGTTCCTCGCCCCCGTACTCCTGCCAGATTCTCA GGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCAGAGGATGAGGCAGA ATATTACTGTGTTCTATGTTACAGCAACCGCTGGTGGTGGTGGAGAACCAAACTGACTGTCC TA
283.	PSMA P2 LCDR1	artificial	aa	DIQMTQSPSSLSASVGDRTVITCRASQGITNYLAWFQQKPKAPKSLIYAASSLQSGVPSKFSGS
284.	PSMA P2 LCDR2	artificial	aa	GSQDFSLTISLQPEDFATYQCQYNSYPITFGQGRLEIK
285.	PSMA P2 LCDR3	artificial	aa	FASQGITNYLA
286.	PSMA P2 VL	artificial	nt	AASSLQ QQNSYPIT GACATCCAGATACCCAGTACCCCTCCTCCCTGTCTGCCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGCTCACAGGCATACCAACTACTGGCTGTTCAGCAGAAAGCCTGGCAAGGCC CTAAGTCCCTGATCTAGCGCGCTCCTCTCTGAGTCCGGCTGCCCTCCAAGTTCTCCGGCTCC GGCTGGCACCGACTTCTCCCTGACCATCTCCTCCCTGCAGCTGAGGACTTCGCCACCTACTA CTGCCAGAGTACAACTCTACCCCTATCACTTCGGCCAGGCAACCCGGCTGGAATCAAG QVQLVESGGGVVQPGRSRLSCAASGFTFSNYVMHWVRQAPGKGLEWVAIIWYDGSNKYYADSVK GRFTISRDNKNTLIYQMNSLRRAEDTAVYYCAGGYNWNYYHYGMDVWGQGTITVTVSS
287.	PSMA P2 H	artificial	aa	NYMH
288.	PSMA P2 HCDR1	artificial	aa	I I WYDGSNKYYADSVK
289.	PSMA P2 HCDR2	artificial	aa	GYNWNYEYHYGMDV
290.	PSMA P2 HCDR3	artificial	aa	CAGTGACGCTGGTCTGAGTCTGGCGGAGGGGTGGTGCAGCCTGGCCGGTCCCTGAGACTGTCTTG CGTGCTCCGGCTTCACTTCTCCAACTACGTGATGCACTGGTGGCGCAGGCTCCAGGCAAGG GACTGGAATGGTGGCCATCATTTGGTACGACGGCTCCAACAAGTACTACGCCGACTCCGTGAAG GGCCGGTTCACCATCTCCCGGACAACTCCAAGAACACCTGTACCTGCAGATGAATCCCTGAG GGCCGAGACACCCCGTGTACTACTGCGCTGGCGGCTACAACCTGGAACACGAGTACCACTACT ACGGCATGACGCTGTGGGCGCAGGGAACCAACCGTGACCGTGTCTTCC
291.	PSMA P2 H	artificial	nt	DIQMTQSPSSLSASVGDRTVITCRASQGITNYLAWFQQKPKAPKSLIYAASSLQSGVPSKFSGS GSGTDFSLTISLQPEDFATYQCQYNSYPITFGQGRLEIKGGGSGGGGGGSGVQLVESG GGVVQPGRSRLSCAASGFTFSNYVMHWVRQAPGKGLEWVAIIWYDGSNKYYADSVKGRFTISR DSKNTLIYQMNSLRRAEDTAVYYCAGGYNWNYYHYGMDVWGQGTITVTVSS
292.	PSMA P2 LH	artificial	aa	DIQMTQSPSSLSASVGDRTVITCRASQGITNYLAWFQQKPKAPKSLIYAASSLQSGVPSKFSGS GSGTDFSLTISLQPEDFATYQCQYNSYPITFGQGRLEIKGGGSGGGGGGSGVQLVESG GGVVQPGRSRLSCAASGFTFSNYVMHWVRQAPGKGLEWVAIIWYDGSNKYYADSVKGRFTISR DSKNTLIYQMNSLRRAEDTAVYYCAGGYNWNYYHYGMDVWGQGTITVTVSS
293.	PSMA P2 LH	artificial	nt	GACATCCAGATGACCCAGTACCCCTCCTCCCTGTCTGCCTCCGTGGCGACAGAGTGACCATCAC

294.	PSMA P2 LH x l2C HL	artificial	aa	CTGCCGGGCTCACAGGGCATCACAACTACCTGGCCTGGTTCCAGCAGAAAGCCTGGCAAGGCCC CTAAGTCCCTGATCTACGCCGCTCTCTCTGAGTCCGGCTGCTTCCAAGTTCTCCGGCTCC GGCTCTGGCACCAGACTTCTCCTGACCATCTCTCCTGCAGCTGAGGACTTGGCCACCTACTA CTGCCAGCAGTACAACTCTACCTTATCACCTTCGGCCAGGCAACCGGCTGAAATCAAGGGCG GAGGGGATCTGGCGCGAGAGAGTGGAGGGGGGATCTCAGTGCAGTGGTGCAGTCTGGC GAGGGTGGTGCAGCTTGGCCGGTCCCTGAGACTGTCTTGCCTGCTGCTCCGGCTTCCATCTC CAATACGTGATGCACTGGGTGGCCAGGCTCCAGCAAGGACTGGAATGGGTGGCCATCATTT GGTACGCGGCTCCAACAAGTACTACGCCGACTCCGTGAAGGGCGGTTCAACATCTCCCGGGAC AACTCCAAGAACACCTGTACCTGCAGATGAATCCCTGAGGGCCGAGGACACCGCTGTACTA CTGCGCTGGCGGTACAACCTGGAACACGAGTACCACTACTACGSCATGACGCTGTGGGGCCAGG GAACACCGTGACCGTGTCTTCC DIQMTQSPSSLSASVGDRTITICRASQGITNYLWFQQKPKAPKSLIYMSLSQSGVPSKFSGS GSGTDFSLTISLSQPEDFATYQCQYNSYPITFGQGTGLEIKGGSGGGGGGQVQLVESG GGVYQPGRSRLRLSCAASGFTFSNYVMHWVRQAPKGLEWVAIIWYDGSNKYYADSVKGRFTISR NSKNTLYLQMNLSLRAEDTAVYYCAGGYNWYEHYHGMVDVWQGTITVTVSSGGGSEVQLVESGG GLVQPGSLKLSCAASGFTFNKYAMNWRQAPKGLEWVARIRSKYNNYATYYADSVKDRFTISR DSDKNTAYLQMNLIKTEDTAVYYCVRHGNEFSYISYWAYWQGLTITVTVSSGGGSGGGSGGGG SQTIVTQEPSTLTVSPGGTITLTCGSTGAVTSGNYPNWVQKPKQAPRGLIIGTKFLAPGTPARF SGSLLGKKAALTLGVQPEDEAEYCYVLWYSNRWVFGGGLTITL
295.	PSMA P2 LH x l2C HL	artificial	nt	GACATCCAGATGACCCAGTCACCTCTCTCCTGTCTGCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGGCTCACAGGGCATCACAACTACCTGGCTGGTTCCAGCAGAAAGCCTGGCAAGGCCC CTAAGTCCCTGATCTACGCCGCTCTCTCTGAGTCCGGCTGCTTCCAAGTTCTCCGGCTCC GGCTCTGGCACCGACTTCTCCTGACCATCTCTCCTGCAGCTGAGGACTTCGCCACCTACTA CTGCCAGCAGTACAACTCTTACCTATCACCTTCGGCCAGGCAACCGGCTGGAATCAAGGGCG GAGGGGATCTGGCGCGAGGAAGTGGAGGGGGGATCTCAGTGCAGCTGGTGCAGTCTGGC GGAGGGTGGTGCAGCTGGCCGGTCCCTGAGACTGTCTTGCCTGCTCCGGCTTCCATCTC CAACTACGTGATGCACTGGTGGCCAGGCTCCAGGCAAGGACTGGAATGGGTGGCCATCATTT GTACACGCGCTCCAACAAGTACTACCGCACTCCGTGAAGGGCGGTTCAACATCTCCCGGAC AACTCCAAGAACACCTGTACTGAGATGAATCCCTGAGGGCCGAGGACACCGCTGTACTA CTGGCTGGCGGCTACAACTGGAACACGATACCACTACCGCATGGACGTGGGGCCAGG GAACACCGTGACCGTGTCTTCCGAGGTGGTGGATCCGAGGTGCAGCTGGTGCAGTCTGGAGGA GGATTGGTGCAGCTTGGAGGTGATGAACTCTCATGTGCAGCTCTGGATTCACTTCAATAA GTACGCGCATGAACCTGGTCCCGAGGCTCCAGGAAAGGTTTGAAITGGTTGCTCGCATAGAA GTAAATATAATAATATGCAACATATATGCCGATTTCAGTGAAGACAGGTTTCAACATCTCCAGA GATGATTCAAAAACACTGCCCTATCTACAAATGAACAACTTGAAACCTGAGGACACTGCCGTGTA CTACTGTGTGAGACATGGGAACCTTCGGTAATAGCTACATATCTACTGGGCTTACTGGGGCCAAAG GGACTCTGGTACCGTCTCCTCAGGTGGTGGTGTCTTGGCGGCGGGCTCCGGTGGTGGTGGT

				TCTCAGACTGTTGTGACTCAGGAACCTTCACCTCACCGTATCACCTGGTGGACAGTCACACTCAC TTGTGGCTCCTCGACTGGGCTGTACATCTGGCAACTACCAAACTGGTCCAAACAAAACCCAG GTCAGGCACCCCGTGTCTAAIAGGTGGACTAAGTTCCTCGCCCCGGTACTCCTGCCAGATTC TCAGGTCCTCGCTTGGAGGCAAGGTGCCCTCACCTCTCAGGGGTACAGCCAGAGGATGAGGC AGAAATATTAATGTTCTATGGTACAGCAACCGCTGGGTGTTCTGGTGGAGGAACCAAACTGACTG TCCTA
296.	PSMA P3 L	artificial	aa	DIQMTQSPSSLSASVGRVTITCRASQGISHYLAWFQQKPKAPKSLIYAASSLQSGVPSKFSGS GSGTDFTLTISSIQPFDFAFYCYQQYNSFPI,TFGGGTKVF,IK
297.	PSMA P3 LCDR1	artificial	aa	RASQGISHYLA
298.	PSMA P3 LCDR2	artificial	aa	AASSLQS
299.	PSMA P3 LCDR3	artificial	aa	QQYNSFPLT
300.	PSMA P3 VL	artificial	nt	GACATCCAGATGACCCAGCTCCTCCTCCTGCTGCTGCCCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGCTCCAGGGCATCTCTCACTACCTGGCTGGTCCAGCAGAAAGCTGGCAAGGCC CTAAGTCCCTGATCTACGCCCTCCTCTCTGCAGTCCGGCTGCCCTTCCAAGTTCTCCGGCTCC GGCTGGCACCGACTTCACCTGACCATCTCCAGCTGCAGCTGAGGACTTCGCCACCTACTA CTGCAGCAGTACAACTCCTTCCCTCTGACCTTCGGCGAGGACCAAGTGGAGATCAAG
301.	PSMA P3 H	artificial	aa	QVQLVESGGGVVQPGRSIRLSAASGFTFSYGMHWVRQAPGKGLDWVAIIWHDGSNKYYADSVK GRFTISRDNSKKTLYIQMNSLRAEDTAVYYCARAWAYDYGYEYFGMDVWGQTTTVTVSS
302.	PSMA P3 HCDR1	artificial	aa	SYGMH
303.	PSMA P3 HCDR2	artificial	aa	LIWHDGSNKYYADSVKG
304.	PSMA P3 HCDR3	artificial	aa	AWAYDYGYEYFGMDV
305.	PSMA P3 H	artificial	nt	CAGGTGAGCTGCTGAGTCTGGCGGAGGGTGGTGCAGCCTGGCCGGTCCCTGAGACTGTCTTG TGCCGCTCCGGCTTCACTTCTCTTACGGCATGCATGGGTGGTGGCGCAGGCTCCAGGCAAGG GACTGGACTGGTGGCCATCATCTGGCAGCAGGCTCCAACAAGTACTACGCCGACTCCGTGAAG GGCGGTTCACCATCTCCGGGACAACCTCAAGAAACCTGTACCTGCAGATGAACCTCCCTGAG GGCGAGGACACCCGCTGTACTGTGCCAGGGCTGGGCTTACGACTACGGCGACTACCGAGT ACTACTCGGCATGGACGTGTGGGCGCAGGGCACCCGTGACAGTGTCTTCC
306.	PSMA P3 LH	artificial	aa	DIQMTQSPSSLSASVGRVTITCRASQGISHYLAWFQQKPKAPKSLIYAASSLQSGVPSKFSGS GSGTDFTLTISSLOPEDFATYCYQQYNSFPLTFGGGTKVEIKGGGGGGGGGGGGGGVQLVESG GGVQPGRSIRLSAASGFTFSYGMHWVRQAPGKGLDWVAIIWHDGSNKYYADSVKGRFTISRD NSKKTLYIQMNSLRAEDTAVYYCARAWAYDYGYEYFGMDVWGQTTTVTVSS
307.	PSMA P3 LH	artificial	nt	GACATCCAGATGACCCAGTCTCCCTCCTCCTGCTGCCCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGCTCCAGGGCATCTCTCACTACCTGGCTGGTCCAGCAGAAAGCTGGCAAGGCC CTAAGTCCCTGATCTACGCCCTCCTCTCTGCAGTCCGGCTGCCCTTCCAAGTTCTCCGGCTCC GGCTGGCACCGACTTCACCTGACCATCTCCAGCTGCAGCTGAGGACTTCGCCACCTACTA CTGCCAGCAGTACAACTCCTTCCCTCTGACCTTCGGCGGAGGGACCAAGTGGAGATCAAGGGCG

				GAGGGGGCTCTGGCGGCGGAGGAAGTGGAGGGGGGATCTCAGGTGCAGCTGGTCGAGTCTGGC GGAGGGGTGGTGACGCTGGCCGGTCCCTGAGACTGTCTTGTGCCCTCCGGCTTACCTTCTC CTCTTACGCATCACTGGTCCGCCAGGCTCCAGGCAAGGACTGGACTGGTGGCGCATCATCT GGCAGCGGCTCCAACAAGTACTAGCCGACTCCGTGAAGGCCGCTTACCATCTCCCGGAC AACTCCAAGAAACCTGTACTGCAGATGAATCCCTGAGGGCCGAGGACACCGCTGTACTA CTGTGCCAGGGCTGGCCCTACGACTACGGGACTACGAGTACTACTTCGGCATGGAAGTGTGGG GCCAGGCAACACCGTGACAGTCTTCC
308.	PSMA P3 LH x I2C HL	artificial	aa	DTQMTQSPSSI.SASVGDRTVTTCRASQGTSHYTAWFQQKPGKAPKSLTYAASSI.QSGVPSKFSGS GSGTDFLT.ISSLQPEDFATYYCQQYNFPLTFGGGTKEIKGGSGSGGGGQVQLVESG GGVQPGRLSLSCAASGFTFSYGMHWVRQAPGKGLDWVAIIWHDGSKYIADSVKGRFTISR NSKLTLYQMNSLRAEDTAVYYCARAWAYDYGYEYFMDVWGQGTITVTVSSGGGSEVQLVES GGGLVQPGSLKLSNAAGFTFNKYAMNWRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTI SRDLSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGTITVTVSSGGGSGGGSGG GGSQTVVTQEPSTVSPGGTVTLTCSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTFKFLAPGTPA RFSGLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGTKLTVL
309.	PSMA P3 LH x I2C HL	artificial	nt	GACATCAGATGACCCAGTCTCCCTCCTCCTGTCTGCCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGCCTCCAGGGCATCTCTACATCCTGGCTGTTCAGCAGAGAGCTGGCAAGGCCC CTAAGTCCCTGATCTACGCCGCTCCTCTCTGCAGTCCGGCTGCTTCCAAGTTCTCCGGCTCC GGCTCTGGCACCGACTTCACCTGACCATCTCCAGCCTGCAGCTGAGGACTTCGCCACCTACTA CTGCCAGCATCAACTCCTTCCCTCTGACCTTCGGCGGAGGACCAAGGTGGAGATCAAGGGCG GAGGGGCTCTGGCGCGGAGGAAGTGGAGGGGCGGATCTCAGGTGCAGCTGGTCGAGTCTGGC GGAGGGTGGTGACGCTGGCCGCTCCCTGAGACTGTCTTGTGCCCTCCGGCTTACCTTCTC CTCTACGGCATGCACTGGGTGCGCCAGGCTCCAGGCAAGGACTGGACTGGGTGGCCATCATCT GGCAGACGGCTCCAACAAGTACTACGCCGACTCCGTGAAGGCCGCTTCAACATCTCCCGGAC AACTCCAAGAAACCTGTACTGCAGATGAATCCCTGAGGCCGAGGACACCGCTGTACTA CTGTGCCAGGGCTGGCCTACGACTACGGGACTACGAGTACTACTTCGGCATGGAAGTGTGGG GCCAGGCAACCGTGACAGTGTCTCCGGAGTGGTGGATCCGAGGTGCAGTGGTCGAGTCT GGAGAGGATTTGGTGACGCTGGAGGGTCATTGAAACTCTCATGTGCAGCTCTGGATTACCTT CAATAAGTACGCCATGAATGGTCCGCCAGGCTCCAGGAAAGGTTTGGAAATGGGTGTCTCGCA TAAGAAGTAAATATAATATGCAACATATTATGCCGATTCAAGTGAAGACAGTTTCAACATC TCCAGAGATGATTCAAAAACACTGCCTATCTACAAATGAACAACCTTGAACACTGAGGACACTGC CGTGTACTACTGTGTGAGACATGGGAACCTCGGTAATAGCTACATACTCTACTGGCTTACTGGG GCCAAGGACTCTGGTCAACGCTCTCTCAGGTGGTGGTGGTCTTGGCGGCGGCTCCGGTGGT GGTGGTCTCAGACTGTGTGACTCAGGAACCTTCACTCACCCTATCACCTGGTGAACAGTCAC ACTCACTGTGGTCTCTGACTGGGCTGTACATCTGGCAACTACCAAACTGGGTCCAAACAA AACAGGTACGGACCCCGTGGTCTAATAGTGGGACTAAGTCTCTGCCGCCGCTACTCTGGC AGATTCTCAGGCTCCCTGCTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGGGA

				TGAGGCAGAAATATTACTGTGTCTTATGGTACAGCAACCGCTGGGTGTTCGGTGGAGGAACCAAAAC TGACTGTCCTA
310.	PSMA P4 L	artificial	aa	DIVMTQTPLSLSVTPGPASISCKSSQSLHSDGKTFLYWYLQKPGQPPQLLIYEVSNRFSGVDP RFGSGSGTDFTLKISRVEAEDVGLYCMQSIQLPLTFGGGKVEIK
311.	PSMA P4 LCDR1	artificial	aa	KSSQSLHSDGKTFLY
312.	PSMA P4 LCDR2	artificial	aa	EVSNRFS
313.	PSMA P4 LCDR3	artificial	aa	MQSIQLPLT
314.	PSMA P4 VL	artificial	nt	GACATCGTGAATACCCAGACCCCTCTGTCCCTGTCTGTGACCCCTGGCCAGCCTGCCTCCATCTC CTGCAAGTCCTCCAGTCCCTGCTGCATCCGACGCTCGACGCAAGACCTTCCTGTACTGGTATCTGCAGA AGCCCGCCAGCCTCTCAGCTGTGATCTACGAGGTGTCCAAACGGTCTCCGGCGTGCCTGAC AGGTTCTGTGGCTCCGGCTCCGGCACCGACTTCAACCTGAAGATCTCCCGGTGGAGGCTGAGGA CGTGGCCCTGTACTACTGATGATGATCCATCCAGTGCCTCTGACCTTCGGCGGAGGACCAAGG TGGAGATCAAG
315.	PSMA P4 H	artificial	aa	QVQLVESGGGVVQPGPSRLRLSCAASGFTFISYGMHWVRQAPGKGLEWVAVISYDGSNKYYADSVK GRFTISRDNKNTLYIQMNSLRAEDTAVYYCARVLVGLALYYNYGMDVWGQTTVTVSS
316.	PSMA P4 HCDR1	artificial	aa	SYGMH
317.	PSMA P4 HCDR2	artificial	aa	VISYDGSNKYYADSVK
318.	PSMA P4 HCDR3	artificial	aa	VLVGLALYYNYGMDV
319.	PSMA P4 H	artificial	nt	CAGTGAGCTGGTCTGAGTCTGGGGAGGGTCTGTGACGCTGGCCGGTCCCTGAGACTGTCTTG CGCGCTCCGGTCACTTCACTCTTACGGCATGCATGGTGCCGCAAGGCTCCAGGCAAGG GACTGGAATGGTGGCTGATCTCTACGACGGCTCCAAAGTACTACGCCGACTCCGTGAAG GGCCGTTCACCATCTCCCGGACAACTCCAAGAACACCTGTACCTGCAGATGAATCCCTGAG GGCCGAGGACACCGCTGTACTGTGCCAGGTGTGTGGCGCTCTGTACTACTACAACT ACTACGGCATGACGTGTGGGCCAGGACACCGTGACAGTGCTTC
320.	PSMA P4 LH	artificial	aa	DIVMTQTPLSLSVTPGPASISCKSSQSLHSDGKTFLYWYLQKPGQPPQLLIYEVSNRFSGVDP RFGSGSGTDFTLKISRVEAEDVGLYCMQSIQLPLTFGGGKVEIKGGGGSGGGSGGSQVQ LVESGGGVVQPGPSRLRLSCAASGFTFISYGMHWVRQAPGKLEWVAVISYDGSNKYYADSVKGRF TISRDNKNTLYIQMNSLRAEDTAVYYCARVLVGLALYYNYGMDVWGQTTVTVSS
321.	PSMA P4 LH	artificial	nt	GACATCGTGAATACCCAGACCCCTCTGTCCCTGTCTGTGACCCCTGGCCAGCCTGCCTCCATCTC CTGCAAGTCTCCAGTCCCTGCTGCATCCGACGGCAAGACCTTCCTGTACTGGTATCTGCAGA AGCCCGCCAGCCTCTCAGCTGTGATCTACGAGGTGTCCAAACGGTCTCCGGCGTGCCTGAC AGGTTCTGTGGCTCCGGCTCCGGCACCGACTTCAACCTGAAGATCTCCCGGTGGAGGCTGAGGA CGTGGCCCTGTACTACTGATGATGATCCATCCAGTGCCTCTGACCTTCGGCGGAGGACCAAGG TGGAGATCAAGGGCGGAGGGGCTCTGGCGCGGAGGAAGTGGAGGGGCGGATCTCAGGTGCAG CTGTGAGTCTGGGGAGGGGTCTGTGACGCTGGCCGCTCCCTGAGACTGTCTTCCGCCGCTC CGGCTTCACTTCTTACGGCATGCACTGGGTGGCCAGGCTCCAGGCAAGGACTGGAAT

322.	PSMA P4 LH x l2C HL	artificial	aa	GGGTGGCGGTGATCTCTTACGACGGCTCCAACAAGTACTACGCCGACTCCGTGAAGGCGCGGTTC ACCATCTCCCGGACAACTCCAAGAACAACCTGTACCTGCAGATGAACCTCCCTGAGGCGCGAGGA CACCGCGGTGACTACTGTGCCAGGTGTGTGGCGCTCTGTACTACTACAACACTACTACGGCA TGGACGTGTGGGCGCAGGGCACACCGTGACAGTGTCTTCC
323.	PSMA P4 LH x l2C HL	artificial	nt	DIVMTQPLSLSVTPGPASISCKSSQSLLSHSDGKTFLYWLKPGQPQLLIYEVSNRFSGVDP RFGSGSDFTLKISRVEAEDVGLYCMQSIQLPLTFGGTKVEIKGGSGSGGSGGGSQVQ LVESSGVVQPGRLSLSCAASGFTFISYGMHVRQAPKGLEWAVISYDGSNKYYADSVKGRF TISRDNKNTIYIQMNSIRAFDTAVYVCARVIVGAIYYNYNYGMVWQQTTVTVSSGGGGSFVQ LVESGGGLVQPGSLKLSAASGFTFNKYAMNVRQAPKGLEWVARIRSKYNNYATYYADSVKD RFTISRDDSKNTAYLQMNLLKTEDTAVYVCVRHGFNSYISYWAYWQGTLVTVSSGGGSGGG GSGGGSQTVVTQEP SLTVSPGGTVTLTSGSSTGAVTSGNYPNWVQKPGQAPRGLIGGTFKFLAP GTPARFSGLLGGKAALTLSGVQPEDEAEYCYVLWYSNRWVFGGTKLTVL GACATCGTATGACCCAGACCCCTCTGTCCCTGTCTGTGACCCCTGGCCAGCCTGCCTCCATCTC CTGCAAGTCCTCCAGTCCCTGTGCACTCCGACGGCAAGACCTTCTGTACTGGTATCTGCAGA AGCCCGCCAGCCTCTCAGCTGTGATCTACGAGGTGTCCAACCGGTCTCCGGCGTGCCTGAC AGGTTCTTGGCTCCGGCTCCGGCACCGACTTCAACCTGAAGATCTCCCGGTGGAGGTGAGGA CGTGGCCTGTACTACTGATGCAGTCCATCCAGTGCCTCTGACCTTCGGCGGAGGACCAAGG TGGAGATCAAGGCGGAGGGGCTCTGGCGGAGGAGGAGTGGAGGGCGGATCTCAGGTGCAG CTGGTCAGTCTGGGGAGGGGTCTGTGAGCCTGGCCGGTCCCTGAGACTGTCTTGCGCCCTC CGCTTCACTTCTTACGGCATGCACTGGTGGCGCAGGCTCCAGGCAAGGACTGGAAT GGTGGCCGTGATCTCTACGACGGCTCCAACAAGTACTACGCCGACTCCGTGAAGGCGCGGTTC ACCATCTCCCGGACAACTCCAAGAACAACCTGTACCTGCAGATGAACCTCCCTGAGGCGCGAGGA CACCCTGTGACTACTGTGCCAGGTGTGTGGCGCTCTGTACTACTACAACACTACTACGGCA TGGAGTGTGGGCGCAGGACACCGTGACAGTGTCTTCCGGAGGTGGTGGATCCGAGGTGCAG CTGGTCAGTCTGGAGAGGATGGTGAGCCTGGAGGTGATGAACCTCTCATGTGCAGCCTC TGGATTACCTTCAATAAGTACCCATGAACCTGGTCCGCCAGGCTCCAGGAAGGTTTGAAT GGTTGCTCGCATAAGAAATAATAATAATATGCAACATATATGCCGATTTCAGTGAAGAC AGGTTCAACATCTCCAGAGATGATTCAAAAACAACCTGCCTATCTACAAATGAACAACCTGAAAC TGAGGACACTGCCGTGTAATACTGTGTGAGACATGGAACTTCGTAATAGTACATATCTTACT GGCTTACTGGGCGCAAGGACTCTGGTCAACCTCTCCTCAGGTGGTGGTCTTGGCGGCGGC GGTCCGGTGGTGGTCTCAGACTGTGTGACTCAGGAACCTTCACTCACCGTATCACCTGG TGGAAAGTCACTCACTTGTGGCTCTCGACTGGGCTGTACATCTGGCAACTACCCAAACT GGTCCCAACAAAAACAGGTGAGGACCCCGTGGTCTAATAGGTGGGACTAAGTCTCGCCCC GGTACTCCTGCCAGATCTCAGGCTCCCTGTGGAGGCAAGGCTGCCCTCACCTCTCAGGGGT ACAGCCAGAGATGAGGCAGAAATATCTGTGTCTATGGTACAGCAACCGCTGGGTGTTCGGTG GAGCAACCAAACTGACTGTCTTA
324.	PSMA P5 L	artificial	aa	AQLTQSPSSLASVGDRTVITCRASQGISSALAWYQQKSGKAPKLLIFDASSLESVPSRFSGS

					GSQDFTLT ISSLPEDFATYYCQFNSYPLTFGGGKVEIK
325.	PSMA P5 LCDR1	artificial	aa		RASQISSALA
326.	PSMA P5 LCDR2	artificial	aa		DASSLES
327.	PSMA P5 LCDR3	artificial	aa		QQENSYPILT
328.	PSMA P5 VL	artificial	nt		GCCATCCAGTGACCCAGAGTCCCTCCCTGCTGCTCCCTGCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGGCTCCAGGGCATCTCTTCGGCCCTGGCTGGTATCAGCAGAAAGTCGGCAAGGCCC CTAAGCTGCTGATCTTCGACGCCCTCTCTTGAATCCGGCGTGCTTCGGGTTCTCCGGCTCT GGTCCGGCACCGACTTCACCCCTGACCATCTCCAGCCTGCAAGCTGAGGACTTCGCCACCTACTA CTGCCAGCAGTTCAACTCTCTACCTCTGACCTTCGGCGGAGGACCAAGTGGAGATCAAG
329.	PSMA P5 H	artificial	aa		QVQLVESGGGVQPGRSRLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVI SYDGNKYYADSVK GRFTISRDN SKNTLYLQMNSLRAEDTAVYYCARAVPWGSRYYYYGMDVWGQTTVTVSS
330.	PSMA P5 HCDR1	artificial	aa		SYAMH
331.	PSMA P5 HCDR2	artificial	aa		VISYDGNKYYADSVKG
332.	PSMA P5 HCDR3	artificial	aa		AVPWGSRYYYYGMDV
333.	PSMA P5 H	artificial	nt		CAGTGACAGTGGTTCGAGTCTGGGGAGGGTCTGTGCAGCCTGGCCGGTCCCTGAGACTGTCTTG CGCCGCTCCGGCTTCACTTCTCTCTTACGCCATGCACTGGTGCGCCAGGCTCCAGGCAAGG GACTGGAATGGTGGCCGTGATCTCTACGACGGCAACAAGTACTACGCCGACTCCCGTGAAG GGCCGTTACCATCTCCGGGACAACCTCAAGAACAACCTGTACTGCAGATGAATCCCTGAG GGTGAAGACACCGCGTGTACTACTGCGCCAGAGCCGTGCTTGGGGTCCCGGTACTACTACT ACGGATGGAGTGTGGGCCAGGGCACCCGTGACAGTGTCTTCC
334.	PSMA P5 LH	artificial	aa		AQLTQSPSSLASVGDRTITCRASQGISSALAWYQQKSGKAPKLLIFDASSLESVPSRFSGS GSQDFTLT ISSLPEDFATYYCQFNSYPLTFGGGKVEIKGGSGSGGGSGGSGVQLVESG GGVQPGRSRLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVI SYDGNKYYADSVKGRFTISR NSKNTLYLQMNSLRAEDTAVYYCARAVPWGSRYYYYGMDVWGQTTVTVSS
335.	PSMA P5 LH	artificial	nt		GCCATCCAGTGACCCAGAGTCCCTCCCTGCTGCTCCCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGGCTCCAGGGCATCTCTCCGCCCTGGCTGGTATCAGCAGAAAGTCCGGCAAGGCCC CTAAGCTGCTGATCTTCGACGCCCTCTCTTGAATCCGGCGTGCTTCGGGTTCTCCGGCTCT GGTCCGGCACCGACTTCACCCCTGACCATCTCCAGCCTGCAGCCTGAGGACTTCGCCACCTACTA CTGCCAGCAGTCAACTCTACCTCTGACCTTCGGCGGAGGGACCAAGTGGAGATCAAGGGCG GAGGGGCTCTGGCGGCGAGGAAGTGGAGGGGCGGATCTCAGGTGCAGCTGGTGCAGTCTGGG GGAGGGTCTGCAGCTTGGCCGCTCCCTGAGACTGTCTTGGCCGCTCCGGCTTCACTTCTC CTCTACGCCATGCACTGGTGGCCAGGCTCCAGGCAAGGACTGGAATGGTGGCCGTGATCT CTTACGCGCAACAAGTACTACGCCGACTCCGTGAAGGGCGGTTCACCATCTCCCGGGAC AACTCAAGAACACCTGTACTGTCAGATGAACCTCCCTGAGGGCTGAGGACACCGCCGTGTACTA CTGCCAGAGCCGTGCTTGGGGTCCCGGTACTACTACGGCATGGACGTGTGGGGCCAGG GCACACCGTGACAGTGTCTTCC

336.	PSMA P5 LH x I2C HL	artificial	aa	AIQLTQSPSSLASVGDRTVITICRASQGISSALAWYQOKSGKAPKLLIFDASSLESQVPSRFSGS GSGTDFTLTISLQPEDFATYICQFNSYPLTFGGTKVEIKGGSGSGGGSGGGSGVQLVESG GGVVQPGFSLRLSCAASGFTFSSYAMHWVRQAPGKLEWAVISYDGNKYAYADSVKGRFTISR NSKNTLYLQMNLSRAEDTAVYICARAVPWGSRYYYGMDVWGQGTIVTVSSGGGSEVQLVESGG GLVQPGGSLKLSCAASGFTFNKYAMNWRQAPGKLEWVARIRSKYNNYATYADSVKDRFTISR DSKNTAYLQMNLIKTEDTAVYICVRHGNFNSYISYWAYWGQGTIVTVSSGGGSGGGSGGGG SQTIVTQEPSTVSPGGTVTLTCGSSTGAVTSNGYPNWVQKPGQAPRGLIGGTFKFLAPGTPARF SGLLGGKAAITLSGVQPEDEAEYYCVLWYSNRWVFGGKLTIVL
337.	PSMA P5 LH x I2C HL	artificial	nt	GCCATCCAGCTGACCCAGAGTCCCTCTCCCTGTCTGCCCTCCGTGGCGACAGAGTGACCATCAC CTGCCGGGCTCCAGGGCATCTCTTCGCCCTGGCTGGTATCAGCAGAAAGTCCGGCAAGGCC CTAAGCTGCTGATCTCGACGCTCTCTCTGGAATCCGGCGTGCTTCCGGTTCTCCGGCTCT GGCTCCGGCACCGACTTCACCTGACCATCTCCAGCTGCAGCTGAGGACTTCGCCACCTACTA CTGCCAGCAGTTCAACTCCTACCTCTGACCTTCGGCGGAGGACCAAGTGGAGATCAAGGGCG GAGGGGCTTGCGGGCGAGAGAGTGGAGGGGCGGATCTCAGSTGCACTGGTCGAGTCTGGG GGAGGGTCTGTGAGCTTGGCCGCTCCCTGAGACTGTCTTGGCCGCTCCGGCTTCACTTCTC CTCTACGCCATGCACTGGGTGCGCAGGCTCCAGGCAAGGACTGGAATGGGTGGCCGTGATCT CCTACGACGCAACAAGTACTACGCCGACTCCGTGAAGGCCGTTTCAACATCTCCCGGGAC AACTCCAAGAACACCTGTACTGCAGATGAACCTCCCTGAGGCTGAGGACACCGCCGTGTACTA CTGGCCAGAGCCGTGCTTGGGCTCCCGTACTACTACGGCATGGACGTGGGGCCAGG GCACACCGTCACAGTGTCTTCGGAGGTGGTGGATCCGAGTGCAGTGGTCGAGTCTGGAGGA GGATTTGGTGACGCTGGAGGTCATTGAAACTCTCATGTGAGCCTCTGGATTCACTTCAATAA GTACGCCATGAACGTGGTCCGCCAGGCTCCAGGAAAGGTTTGGAAATGGGTGCTCGCATAGAA GTAATAATAATAATTATGCAACATATTATGCCGATTCAAGTGAAGACAGGTTCCACATCTCCAGA GATGATTCAAAAACACATGCCATCTACAAATGAACAACTTGAAACTGAGGACACTGCCGTGTA CTACTGTGTGAGACATGGAACTTCGGTAATAGTACATATCTACTGGCTTACTGGGGCCAAAG GGACTCTGTCACGCTCTCTCAGGTGGTGGTGGTCTGGCGGCGGGCTCCGGTGGTGGTGGT TCTCAGACTGTGTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGGACAGTCACACTCAC TTGTGGCTCCTCGACTGGGCTGTACATCTGGCAACTACCAAACTGGGTCCAAACAAAACAG GTCAGGACCCCGTGGTCTAAATAGTGGGACTAAGTTCCTCGCCCCGGTACTCTCTGCCAGATT TCAGGCTCCCTGCTTGGAGCAAGGTCCTCACCTCTCAGGGGTACAGCCAGGATGAGGC AGAATATTACTGTGTCTATGTTACAGCAACCGCTGGGTGGTGGAGGAAACCAACTGACTG TCCTA
338.	PSMA D1 L	artificial	aa	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWFQQKPGQAPRLLIYDASNRAITGIPARFSGS GSGTDFTLTISLQPEDFAVYICQQRSNWLMYTFQGQTKLEIK
339.	PSMA D1 LCDR1	artificial	aa	FASQSVSSYLA
340.	PSMA D1 LCDR2	artificial	aa	DASNRAIT
341.	PSMA D1 LCDR3	artificial	aa	QQRSNWLMT

342.	PSMA D1 VL	artificial	nt	GAGATCGTGTGACCCAGTCCCTGCCACCCCTGTCTCTGTCTCCCGGGGAGAGAGCCACCCCTGAG CTGCCGGCCTCCAGTCCGTGTCTCTACCTACCTGGCTGGTCCAGCAGAACCTGGACAGGCCC CTAGGCTGTGATCTACGACGCCCTCAACAGGGCTACCGGCATCCCTGCCGGTCTCCGGCTCT GGTCCGGCACCGACTTCACCCCTGACCATCTCCAGCCTGGAACCTGAGGACTTCGCCGTGTACTA CTGCCAGCAGCGGTCCAACTGGCTGATGTATACCTTCGGCCAGGACCACCAAGCTGGAATCAAG
343.	PSMA D1 H	artificial	aa	EYQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWARQMPGKLEWMGIIYPGDS DTRYSPSFQ GQVTISADKSI STAYLQWSSLKASDTAMYCYCSAANSSHWFYDLWGRGTLVTVSS
344.	PSMA D1 HCDR1	artificial	aa	SYWIG
345.	PSMA D1 HCDR2	artificial	aa	IYPGDS DTRYSPSFQ
346.	PSMA D1 HCDR3	artificial	aa	ANSSHWFYDL
347.	PSMA D1 H	artificial	nt	GAAGTGAGCTGGTGACGTCTGGCGCCGAAAGTGAAGAACCCCGGCGAGTCCCTGAAGATCTCCTG CAAGGGCTCCGGTACTCTTACCTCTCTGATCGGATCGGCTGGCCAGGCAGATGCCAGGCAAGG GCCTGGAATGGATGGGCATCATCTACCTGGGACTCCGACACCCGGTACTCTCCAGCTTCCAG GGCAGGTGACCATCTCTGCCGACAAGTCCATCTCCACCGCTACCTGCAGTGTCTCCCTGAA GGCTCCGACACCGCATGTACTATTGTCTCCGCGCCAACTCCTCCACTGCTACTTCGACCTGT GGGAGAGGCCCTGGTGACCGTGTCTTCC
348.	PSMA D1 LH	artificial	aa	EIVLTQSPATLSLSPGERATLSRASQSVSSYLAWFQQKPGQAPRLIYDASNRTGIPARFSGS GSGTDFTLTISLLEPEDFAVYYCQQRNWLMTFGQGTGLEIKGGGGSGGGGGSEVQLVQS GAEVKKPGEISLKI SC KSGYSF"SYWIGWARQMPGKLEWMGIIYPGDS DTRYSPSFQGVITISA DKSISTAYLQWSSLKASDTAMYCYCSAANSSHWFYDLWGRGTLVTVSS
349.	PSMA D1 LH	artificial	nt	GAGATCGTGTGACCCAGTCCCTGCCACCCCTGTCTCTGTCTCCCGGGGAGAGAGCCACCCCTGAG CTGCCGGCCTCCAGTCCGTGTCTCTTACCTACCTGGCTGGTTCAGCAGAACCTGGACAGGCCC CTAGGCTGTGATCTACGACGCCCTCAACAGGGCTACCGGCATCCCTGCCGGTCTCCGGCTCT GGTCCGGCACCGACTTCACCTGACCATCTCCAGCTGGAACCTGAGGACTTCGCCGTGTACTA CTGCCAGCAGCGGTCCAATGGCTGATGTATACCTTCGGCCAGGCAACCAAGCTGGAATCAAGG CGGAGGGGATCTGGGGCGGAGGAAGTGGAGGGGGCGGATCCGAAGTCCAGCTCCGGTGTACTCT GGCGCGAAGTGAAGAACCCGGCGAGTCCCTGAAGATCTCTGCAAGGCTCCGGGTACTCTCTT CACCTCTACTGGATCGGTGGCCAGGCAGATGCCAGGCAAGGCTGGAATGGATGGGCATCA TCTACCTGGCGACTCCGACACCCGGTACTCTCCAGCTTCAGGGCCAGGTACCATCTCTGCC GACAAAGTCCATCTCCACCGCTACCTGCAGTGTCTCTCCCTGAAGGCTCCGACACCGCATGTA CTATTGCTCCGCGCCAACTCCTCCACTGCTACTTCGACCTGTGGGCGAGAGGACCCCTGGTGA CCGTGCTTCC
350.	PSMA D1 LH x I2C HL	artificial	aa	EIVLTQSPATLSLSPGERATLSRASQSVSSYLAWFQQKPGQAPRLIYDASNRTGIPARFSGS GSGTDFTLTISLLEPEDFAVYYCQQRNWLMTFGQGTGLEIKGGGGSGGGGGSEVQLVQS GAEVKKPGEISLKI SC KSGYSF"SYWIGWARQMPGKLEWMGIIYPGDS DTRYSPSFQGVITISA DKSISTAYLQWSSLKASDTAMYCYCSAANSSHWFYDLWGRGTLVTSSGGGGSEVQLVESGGGLVQ PGGSLKLSCAASGFTFNKYAMNWNVRQAPGKLEWVARIRSKYNNYATYYADSVKDRFTISRDDSK

					GOVTSADKSI STAYLQWNSLKTS DTAMYYCATANPSYWF DLWGRGLVTVSS
358.	PSMA D2 HCDR1	artificial	aa		SYWIG
359.	PSMA D2 HCDR2	artificial	aa		LIYPGSDTRYSPSFQG
360.	PSMA D2 HCDR3	artificial	aa		ANPSYWF DL
361.	PSMA D2 H	artificial	nt		GAAGTGAGTGGTGACGTCTGGCGCCGAAGTGAACCCCTGGCGAGTCCCTGAAGATCTCCTG CAAGGGCTCCGGCTACACCTTACCTTCTACTGGATCGGTGGTGCGCAGATCGCTGGCAAG GCCCTGAGTGATGGCATCATCTACCTGGGACTCCGACACCCGGTACTCTCCAGCTTCCAG GGCAGGTGACCTTCTCCGCGACAAAGTCCATCTCCACCGCTACCTGCAGTGAACCTCCCTGA AACCTCCGACACCGCATGTACTATTGGCCACCCGCAACCTAGCTACTGTACTTCGACCTGT GGGACAGGACCCCTGGTGACCGTGCTCTCC
362.	PSMA D2 LH	artificial	aa		EIVLTQSPATLSLSPGERATLSCRASQSVSYLAWYQQKPGQAPRLLIYDASNRAIGIPARFSGS GSGTDFLTLSLEPEDFAVYYCQQRSDWIMYTFQGKLEIKGGSGSGSGGGSEVQLVQS GAEVKTPEGLKISCKGSGYTFTSYWIWVRQMPGKGPWMGI IYPGSDTRYSPSFQGVTFSA DKSISTAYLQWNSLKTS DTAMYYCATANPSYWF DLWGRGLVTVSS
363.	PSMA D2 LH	artificial	nt		GAGATCGTGCTGACCCAGTCCCTGCCACCTGTCTGTCTCCCGGCGAGAGCCACCCCTGAG CTGCCGGCTCCAGTCCGTGTCTCTACCTGGCTGGTATCAGCAGAGCCCTGGACAGGCC CTAGGTGTGTATACGACGCTCCAAAGGCTACCGGCATCCCTGCCGGTCTCCGGCTCT GGCTCCGGACCGACTTACCTGACCATCTCCAGCTGGAACCTGAGGACTTCCGCTGTACTA CTGCCAGACGGTCCGACGTGATGATACCTTCCGCGAGGACCAAGTGGAAATCAAGG GCGGAGGGGATCTGGCGCGGAGGAGTGGAGGGGGCGGATCCGAAAGTGCAGTGGTGAGTCT GGCGCGAAGTGAACCCCTGGCGAGTCCCTGAAGATCTCTGCAAGGCTCCGGCTACACCTT CACCTCTACTGGATCGGTGGTGCGCAGATGCTTGCAAGGCCCTGAGTGGATGGGCATCA TCTACCTGGCGACTCCGACACCCGGTACTTCCCAGCTTCCAGGCGCAGGTGACCTTCTCCGCC GACAAGTCCATCTCCACCGCTACCTGCAGTGGAATCCCTGAAACCTCCGACACCGCCATGTA CTATTGCGCCACCGCAACCTAGCTACTGTGTACTTCCACCTGTGGGCGAGAGCCACCCCTGGTGA CCGTGCTTCC
364.	PSMA D2 LH x I2C HL	artificial	aa		EIVLTQSPATLSLSPGERATLSCRASQSVSYLAWYQQKPGQAPRLLIYDASNRAIGIPARFSGS GSGTDFLTLSLEPEDFAVYYCQQRSDWIMYTFQGKLEIKGGSGSGSGGGSEVQLVQS GAEVKTPEGLKISCKGSGYTFTSYWIWVRQMPGKGPWMGI IYPGSDTRYSPSFQGVTFSA DKSISTAYLQWNSLKTS DTAMYYCATANPSYWF DLWGRGLVTVSSGGGSEVQLVSGGGIVQ PGSLKLSCAAAGTFFNKYAMNVRQAPGKGLEWVARIRSKYNNYATYADSVKDRFTI SRDSDK NTAYLQMNLLKTEDTAVYYCVRHGNFGNSY ISYWAYWGQGLTVTVSSGGGSGGGSGGGSQTV VTQEPSTVSPGGTVTLTCCSSTGAVTSGNYPNWQKPGQAPRLIIGGTFKFLAPGTPARFSGSL LGKKAALTLSCVQPEDEAEYCYVLYWYNRWVFGGKTLTVL
365.	PSMA D2 LH x I2C HL	artificial	nt		GAGATCGTGCTGACCCAGTCCCTGCCACCTGTCTGTCTCCCGGCGAGAGCCACCCCTGAG CTGCCGGGCTCCAGTCCGTGTCTCTCTACCTGGCTGGTATCAGCAGAGCCCTGGACAGGCC CTAGGCTGTGTATACGACGCTCCAAAGGCTACCGGCATCCCTGCCGGTCTCCGGCTCT

			GGTCCGGCACCGACTTCACCCCTGACCATCTCCAGCCTGGAACCTGAGGACTTCGCCCTGTACTA CTGCCAGACCGGTCCGACTGGCTGATGTATACCTTCGGCCAGGSCACCAAGCTGGAATCAAGG GGGAGGGGGATCTGGCGCGCAGGAAGTGAGGGGGGGATCCGAAGTGCAGCTGGTGCAGTCT GGCGCGAAGTGAACACCCCTGGCGAGTCCCTGAAGATCTCTGCAAGGCTCCGGCTACACCTT CACCTTACTGGATCGGTGGGTGGCCAGATGCTGGCAAGGCCCTGAGTGGATGGGCATCA TCTACCCGTGCGACTCCGACACCCGCTACTCTCCAGTTCAGGGCCAGGTGACCTTCTCCGCC GACAGTCCATCTCCACCGCTACCTGCAGTGAACCTCCCTGAAAACCTCCGACACCCGCCATGTA CTATTGCCACCGCAACCTAGTACTAGTACTTCGACCTGTGGGCGAGGACCCCTGGTGA CCGTCTTCCGAGGTGGTGGATCCGAGTGCAGCTGGTGCAGTCTGGAGGAGTCTGGTGCAG CCTGGAGGTCAATTGAACTCTCATGTGCAGCTCTGGATTCACTTCAATAAGTACGCCATGAA CTGGTCCGCCAGGCTCCAGGAAGGTTTGAATGGTGTCTCGCATAGAAGTAAATATAATA ATTATGCAACATATTATGCCGATTCACTGAAAGACAGGTTCAACCATCTCCAGAGATGATCAAAA AACATGCCATCTACAAATGAACAACTTGAACAACTGAGGACACTGCCGTGTACTACTGTGTGAG ACATGGAACTTCGGTAATAGCTACATATCTACTGGGCTTACTGGGCCAAGGACTCTGGTCA CCGTCTCTCAGGTGGTGGTCTGGCGCGGGCTCCGGTGGTGGTCTCAGACTGTT GTGACTCAGGAACCTTCACTCACCGTATCACCTGGTGAACAGTCACTCACTTGTGGTCTCTC GACTGGGCTGTACATCTGGCAACTACCAAACTGGTCCAAACAAACAGGTCAAGCACCCC GTGGTCTAATAGTGGGACTAAGTCTCTCGCCCCGGTACTCTGCCAGATTCTCAGGCTCCCTG CTTGAGGCAAGGCTGCCCTCACCTCTCAGGGGTACAGCCAGAGATGAGGCAGATATTACTG TGTTCTATGTTACAGCAACCGCTGGGTGTTCCGTGGAGGAACCAACTGACTGCTCTA
			ATGAAGACTTGGGTAAATAATCGTATTTGGAGTTGCCACCTCTGCTGTGCTTGCCTTATTGGTGAT GTGCAATTGTCTTACGCCCTTCAAGAGTTCAATACTCTGAAGAAAATACAATGAGAGCACTCACAC TGAAGGATATTTTAAATGGAACATTTTCTTATAAAACATTTTTCCAAACCTGGATTTCAGGACAA GAATATCTTCACTCAATCTGCAGATAACAATATAGTACTTTATAATTTGAAACAGGACAAATCATA TACCAATTTTGAGTAATAGAACCATGAAAAGTGTGAATGCTTCAATTACGGCTTATCACCTGATC GGCAATTTGTATATCTAGAAAAGTGTATTTCAAAGCTTTGGAGATCTTACACAGCAACATAT TACATCTATGACCTTAGCAATGGAGAATTTGTAAGAGGAAATGAGCTTCCCTCGTCCAATTCAGTA TTTATGCTGTCGCTGTTGGGAGTAAATTAGCATATGTCTATCAAAACAATATCTATTTGAAAC AAAGACCAGGAGATCCACCTTTTCAAAATAACATTTAATGGAAGAGAAAATAAAATATTTAATGGA ATCCCAGACTGGGTTTATGAAGAGGAAATGCTTGTCTACAAAATATGCTCTCTGGTGGTCTCTCTAA TGGAATAATTTTGGCATATGCGGAATTTAATGATACGGATATACCAGTTATGCTCTATTTCCCTATT ATGGCGATGAACAATATCTAGAACAAATAATATCCATACCCAAAGGCTGGAGCTAAGAATCCC GTTGTCGGATATTTATTCATACCACTTACCCTCGGTATGTAGGTCCCGAGGAAGTGCCTGT TCCAGCAATGATAGCCTCAAGTGATTTATTTTCAAGTTGGTTCAGTGGGTACTGATGAACGAG TATGTTTGCAGTGGCTAAAAGAGTCCAGAAATGTTTCGGTCTGTCTATGTGACTTCAGGGAA GACTGGCAGACATGGGATTTGCCAAAGACCCAGGAGCATATAGAAGAAAGCAGAACTGGATGGGC TGGTGGATTCTTTGTTTCAACACCAAGTTTTCAGCTATGATGCCATTTTCGTACTACAAAATATTTTA
366.	human FAP α construct	artificial	nt

367.	human FAP α construct	artificial	aa	GTGACAAAGGATGGCTACAAAACATATTCACATATATCAAAGACACACTGTGGAAAATGCTATTCAAATTT ACAAAGTGCAAGTGGGAGGCCATAAATATATTCAGAGTAACACAGGATTCACTGTTTATTATTCTAG CAATGAATTTGAAGAATACCTTGGAAGAAGAAACATCTACAGAAATTAGCATTTGAAGCTATCCCTC CAAGCAAGAAGTGTGTACTTGCATCTAAGGAAAGAAAGGTGCCAATATTACACAGCAAGTTTC AGCGACTACGCCAAGTACTATGCACCTTGCTGTACGGCCAGGCATCCCAATTTCCACCCCTTCA TGATGGACGACTGATCAAGAAATTAATAATCCTGGAAGAAACAAAGAAATGGAAAATGCTTTGA AAAATATCCAGCTGCCTAAAGAGGAAATTAAGAACTTGAAGTAGATGAATTAATCTTATGGTAC AAGATGATTTCTCTCCTCAATTTGACAGATCAAAAGAGTATCCCTTGCTAATTCAGTGTATGG TGGTCCCTGCAGTCAGAGTAAAGTCTGTATTTGCTGTAAATGGATATCTTATCTTGAAGTA AGGAAGGATGTCATTGCCCTGGTGGATGGTCGAGGAACAGCTTTCCAAGGTGACAAACTCCTC TATGCAGTGTATCGAAAAGTGGTGTATTATGAAGTTGAAGACCAGATTACAGCTGTGAGAAAAT CATAGAAATGGGTTTCAATGATGAAAAAAGAAATAGCCATATGGGCTGGTCTATGGAGGATACG TTTCATCACTGGCCCTTGCACTCTGGAACCTGGTCTTTTCAAATGTGGTATAGCAGTGGCTCCAGTC TCCAGCTGGGAAATATTACGGCTGTCTACACAGAGAGATTATGGTCTCCCAACAAGGATGA TAATCTTGAGCACTATAAGAAATCAACTGTGATGGCAAGACAGAAATATTCAGAAATGTAGACT ATCTTCTCATCCACGGAACAGCAGATGATAATGTGCACCTTTCAAACCTCAGCACAGATTGCTAAA GCTCTGGTTAATGACAAAGTGGATTCCAGGCAATGTGGTACTCTGACCAGAACCCACGGCTTATC CGGCCCTGCCACGAACCACTTATACACCCACATGACCCCACTTCTCTAAAGCAGTGTCTCTCTGT CAGACTAA
368.	soluble fusion protein of h FAP α and m Lag3	artificial	nt	MKTWVKIVFVGVATSAVALLVMCIVLRPSRVHENSENTRALTLDKILNGTFSYKTFPPNWSIGQ EYLHQSADNNIVLYNIETGQSYTILSNRTMKSVAASNYGLSPDRQFVYLES DY SKLWRY SYTATY YIYDL SNGE FVRGNELPRPIQYLCWSPVGSKLAYVYQNNIY LKQRPDPFPQITFNGRENKIFNG IPDWVYEEEMLATKYALWSPNGKFLAYAEFNDDIPIVIAYSYGYDEQYPRITINIPYPRKAGKNP VVRIFIIDITYPAYVGPQEVVPAMIASSDYFSWLTWVTDERVCLQWLKRVQNVSVLSICDFRE DWQTDWCPKTQEHIESRTGWAGGFVSTPVSFYDAISYKIFSDKDGKXHYIKDITVENAIQI TSGKWEAINIFRVTQDSLFSYSSNEFEYFGRNRIYRISIGSYPPSKKCVTCHLRKERCQYVTASE SDYAKYVALVCYCPGIPISITLHDGRTDQEI KILEENKELENALKNIQLPKEIKKLEVDEITLWY KMTILPPQFDRSKKYPILLIQVYGGPCSQSVRSVFAVNWISYASKEGMVIALVDGRGTAFQGGKLL YAVYRKLGVYEVEDQITAVRKFIEMGFIDEKRIAIWGSYGGYVSSALASGTGLFKCGIAVAPV SSWEYAYASVYTERFMGLPTKDDNLEHYKNSTVMARAEYFRNVLDYLLIHGTADDNVHFQNSAQIAK ALVNAQVDFQAMWYSQDNHGLSGLSTNHLYTHMTHFLKQCFSLSD

DEMANDES OU BREVETS VOLUMINEUX

**LA PRÉSENTE PARTIE DE CETTE DEMANDE OU CE BREVETS
COMPREND PLUS D'UN TOME.**

CECI EST LE TOME __1__ DE __2__

NOTE: Pour les tomes additionels, veuillez contacter le Bureau Canadien des Brevets.

JUMBO APPLICATIONS / PATENTS

**THIS SECTION OF THE APPLICATION / PATENT CONTAINS MORE
THAN ONE VOLUME.**

THIS IS VOLUME __1__ OF __2__

NOTE: For additional volumes please contact the Canadian Patent Office.

We Claim:

1. A method for the selection of bispecific single chain antibodies comprising a first binding domain specifically binding to an epitope of CD3 comprising antigen regions with a distance of more than 60A from a reference C-atom and a second binding domain specifically binding to the extracellular domain of prostate-specific membrane antigen (PSMA) within a distance of less than 60A from the reference C-atom, the method comprising the steps of:
 - (a) providing at least three types of host cells expressing
 - (i) the wt human extracellular domain of PSMA on the cell surface wherein the wt human extracellular domain of PSMA comprises SEQ ID NO: 447 in at least a first type of host cell;
 - (ii) a mutated form of the wt human PSMA on the cell surface, wherein the amino acid residues at positions 140, 169, 191, 308, 334, 339, 344, 624, 626, 716, 717 and 721 are mutated to the corresponding amino acid residues of the wt rat PSMA in at least a second type of host cell; and
 - (iii) the rat wt extracellular domain of PSMA on the cell surface in at least a third type of host cell;
 - (b) contacting each type of host cells (i), (ii) and (iii) of step (a) with the bispecific single chain antibodies comprising the first binding domain binding to an epitope of CD3 and the second binding domain binding to the extracellular domain of PSMA, and effector T cells; and
 - (c) identifying and isolating the bispecific single chain antibodies that mediate lysis of host cells expressing wt human extracellular domain of PSMA on the cell surface according to (a)(i) and of host cells expressing mutated form of the wt human PSMA on the cell surface according to (a)(ii) but not of host cells expressing the rat wt extracellular domain of PSMA on the cell surface according to a(iii).

2. The method of claim 1, wherein the first binding domain binds to CD3 epsilon (CD3 ϵ) of human and *Callithrix jacchus*, *Saguinus oedipus*, or *Saimiri sciureus*.

3. The method of claim 2, wherein the first binding domain comprises a VL region comprising CDR-L1, CDR-L2 and CDR-L3 wherein CDR-L1, CDR-L2 and CDR-L3 are:
 - (a) CDR-L1 as depicted in SEQ ID NO. 27, CDR-L2 as depicted in SEQ ID NO. 28 and CDR-L3 as depicted in SEQ ID NO. 29;
 - (b) CDR-L1 as depicted in SEQ ID NO. 117, CDR-L2 as depicted in SEQ ID NO. 118 and CDR-L3 as depicted in SEQ ID NO. 119; or

- (c) CDR-L1 as depicted in SEQ ID NO. 153, CDR-L2 as depicted in SEQ ID NO. 154 and CDR-L3 as depicted in SEQ ID NO. 155.
4. The method of claim 2, wherein the first binding domain comprises a VH region comprising CDR-H 1, CDR-H2 and CDR-H3, wherein CDR-H 1, CDR-H2 and CDR-H3 are:
- (a) CDR-H1 as depicted in SEQ ID NO. 12, CDR-H2 as depicted in SEQ ID NO. 13 and CDR-H3 as depicted in SEQ ID NO. 14;
 - (b) CDR-H1 as depicted in SEQ ID NO. 30, CDR-H2 as depicted in SEQ ID NO. 31 and CDR-H3 as depicted in SEQ ID NO. 32;
 - (c) CDR-H1 as depicted in SEQ ID NO. 48, CDR-H2 as depicted in SEQ ID NO. 49 and CDR-H3 as depicted in SEQ ID NO. 50;
 - (d) CDR-H1 as depicted in SEQ ID NO. 66, CDR-H2 as depicted in SEQ ID NO. 67 and CDR-H3 as depicted in SEQ ID NO. 68;
 - (e) CDR-H1 as depicted in SEQ ID NO. 84, CDR-H2 as depicted in SEQ ID NO. 85 and CDR-H3 as depicted in SEQ ID NO. 86;
 - (f) CDR-H1 as depicted in SEQ ID NO. 102, CDR-H2 as depicted in SEQ ID NO. 103 and CDR-H3 as depicted in SEQ ID NO. 104;
 - (g) CDR-H1 as depicted in SEQ ID NO. 120, CDR-H2 as depicted in SEQ ID NO. 121 and CDR-H3 as depicted in SEQ ID NO. 122;
 - (h) CDR-H1 as depicted in SEQ ID NO. 138, CDR-H2 as depicted in SEQ ID NO. 139 and CDR-H3 as depicted in SEQ ID NO. 140;
 - (i) CDR-H1 as depicted in SEQ ID NO. 156, CDR-H2 as depicted in SEQ ID NO. 157 and CDR-H3 as depicted in SEQ ID NO. 158; or
 - (j) CDR-H1 as depicted in SEQ ID NO. 174, CDR-H2 as depicted in SEQ ID NO. 175 and CDR-H3 as depicted in SEQ ID NO. 176.
5. The method according to any one of claims 2 or 3, wherein the first binding domain comprises a VL region, wherein the VL region is a VL region as depicted in SEQ ID NO. 35, 39, 125, 129, 161 or 165.
6. The method according to any one of claims 2 or 4, wherein the first binding domain comprises a VH region, wherein the VH region is a VH region as depicted in SEQ ID NO. 15, 19, 33, 37, 51, 55, 69, 73, 87, 91, 105, 109, 123, 127, 141, 145, 159, 163, 177 or 181.

7. The method according to any one of claims 2 to 6, wherein the first binding domain comprises a VL region and a VH region, wherein the VL region and the VH region are:
- (a) a VL region as depicted in SEQ ID NO. 17 or 21 and a VH region as depicted in SEQ ID NO. 15 or 19;
 - (b) a VL region as depicted in SEQ ID NO. 35 or 39 and a VH region as depicted in SEQ ID NO. 33 or 37;
 - (c) a VL region as depicted in SEQ ID NO. 53 or 57 and a VH region as depicted in SEQ ID NO. 51 or 55;
 - (d) a VL region as depicted in SEQ ID NO. 71 or 75 and a VH region as depicted in SEQ ID NO. 69 or 73;
 - (e) a VL region as depicted in SEQ ID NO. 89 or 93 and a VH region as depicted in SEQ ID NO. 87 or 91;
 - (f) a VL region as depicted in SEQ ID NO. 107 or 111 and a VH region as depicted in SEQ ID NO. 105 or 109;
 - (g) a VL region as depicted in SEQ ID NO. 125 or 129 and a VH region as depicted in SEQ ID NO. 123 or 127;
 - (h) a VL region as depicted in SEQ ID NO. 143 or 147 and a VH region as depicted in SEQ ID NO. 141 or 145;
 - (i) a VL region as depicted in SEQ ID NO. 161 or 165 and a VH region as depicted in SEQ ID NO. 159 or 163; or
 - (j) a VL region as depicted in SEQ ID NO. 179 or 183 and a VH region as depicted in SEQ ID NO. 177 or 181.
8. The method according to claim 7, wherein the first binding domain comprises an amino acid sequence of SEQ ID NOs: 23, 25, 41, 43, 59, 61, 77, 79, 95, 97, 113, 115, 131, 133, 149, 151, 167, 169, 185 or 187.

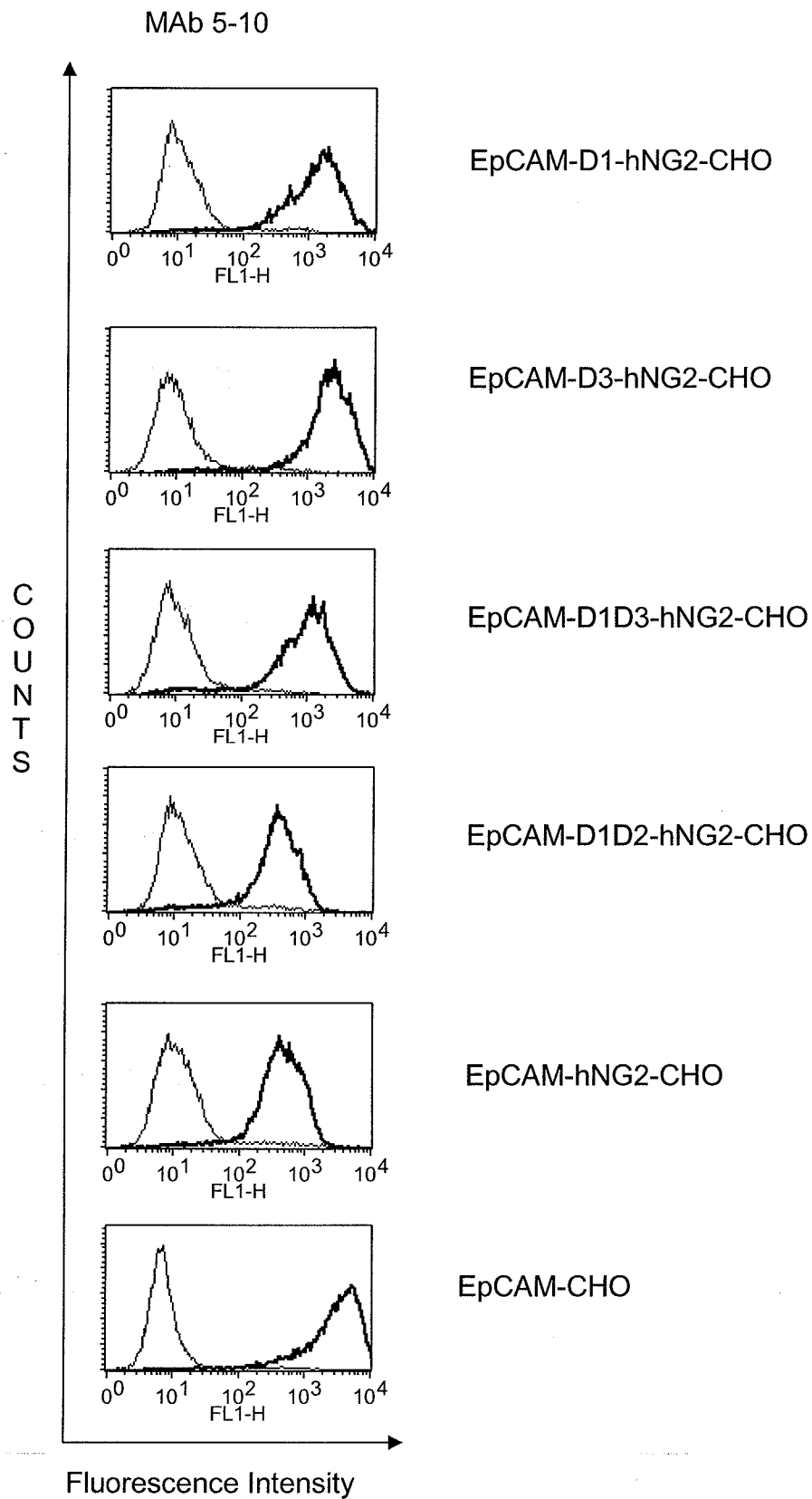
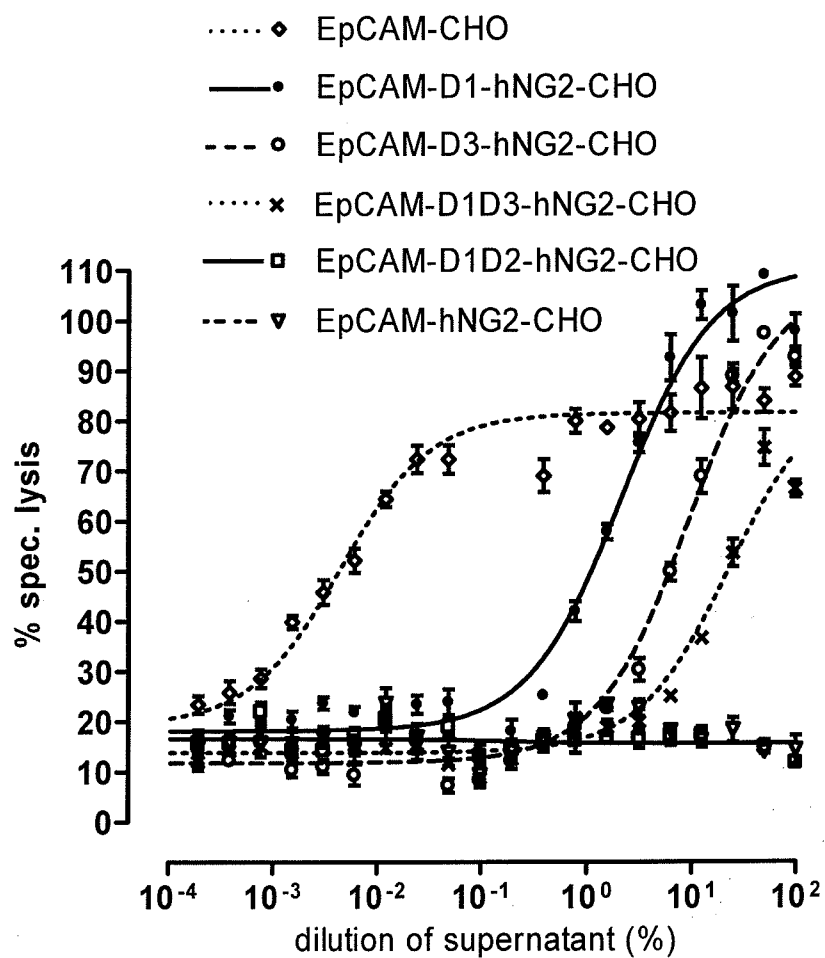
Figure 1

Figure 2

[illegible]

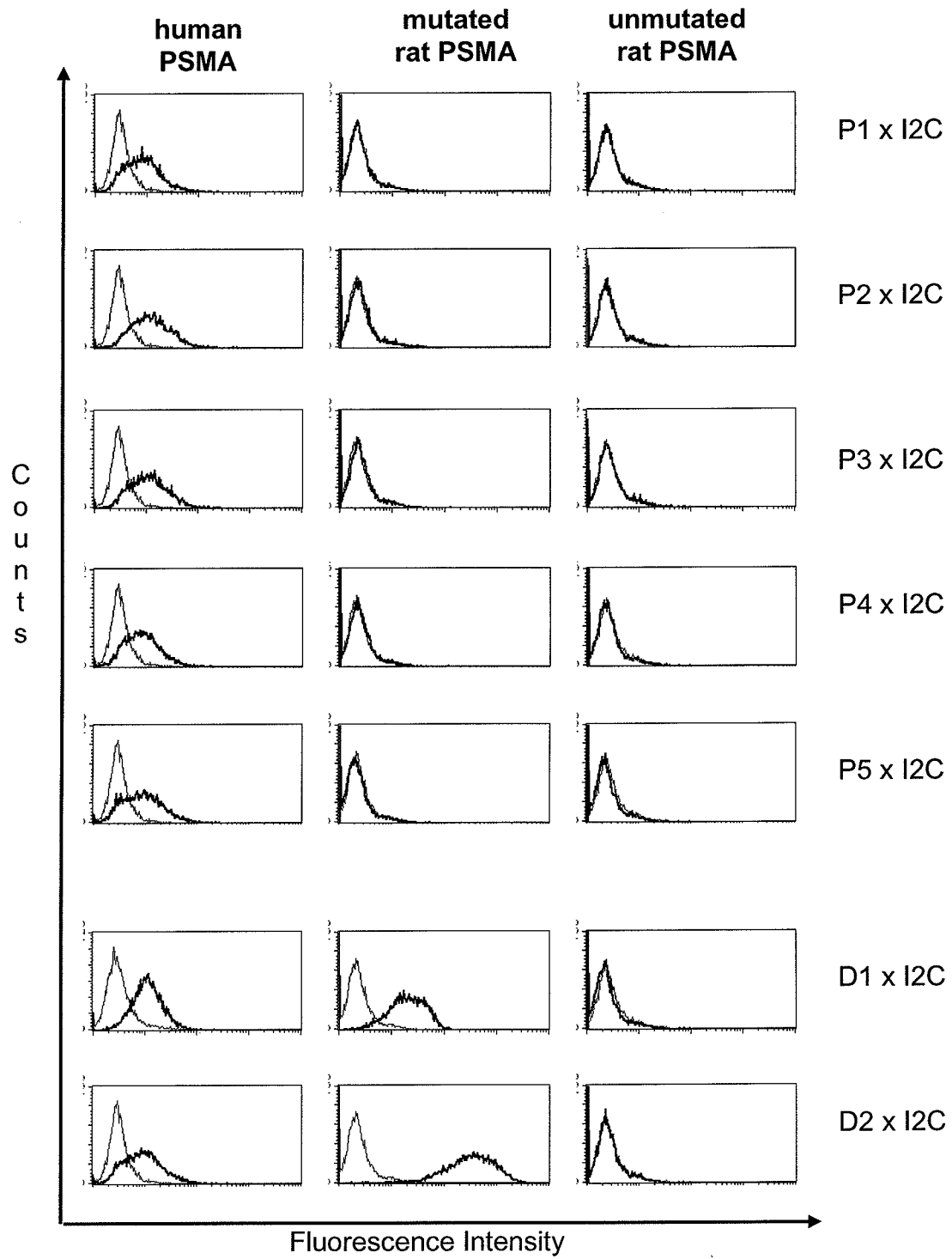
Figure 4

Figure 5

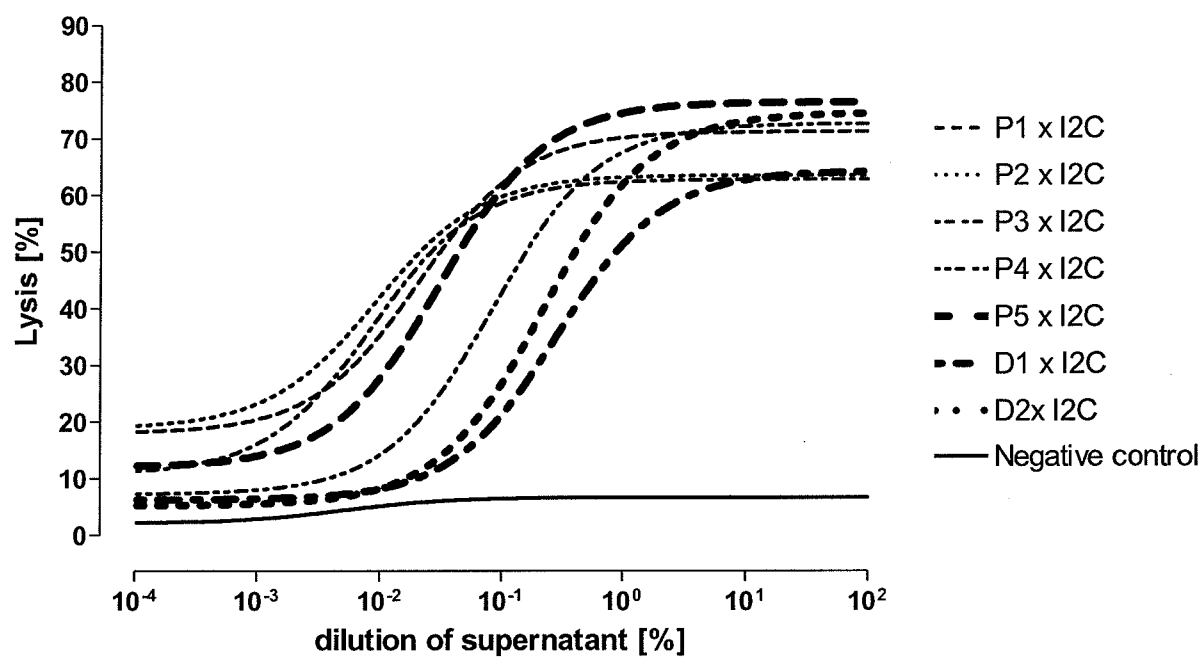


Figure 6

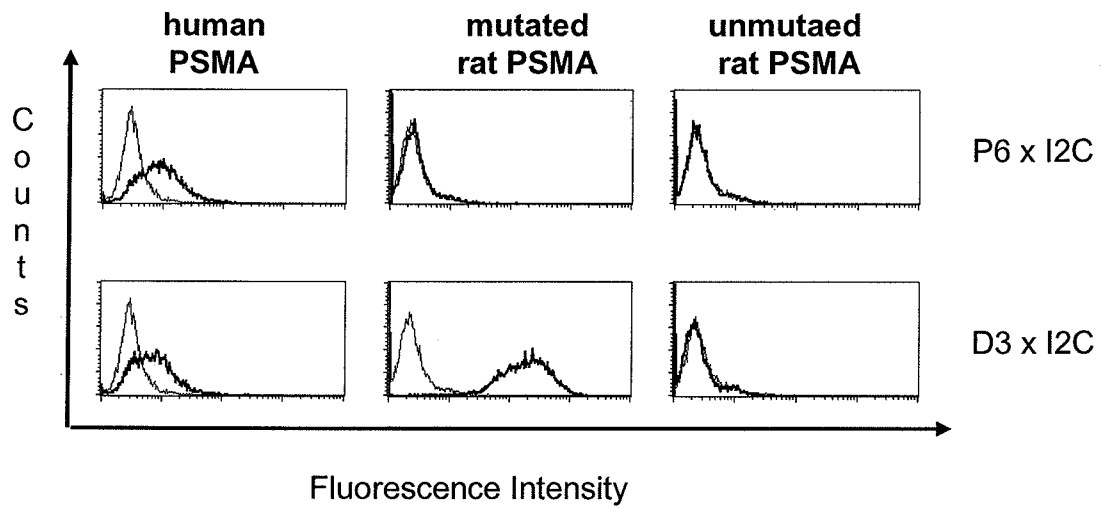


Figure 7

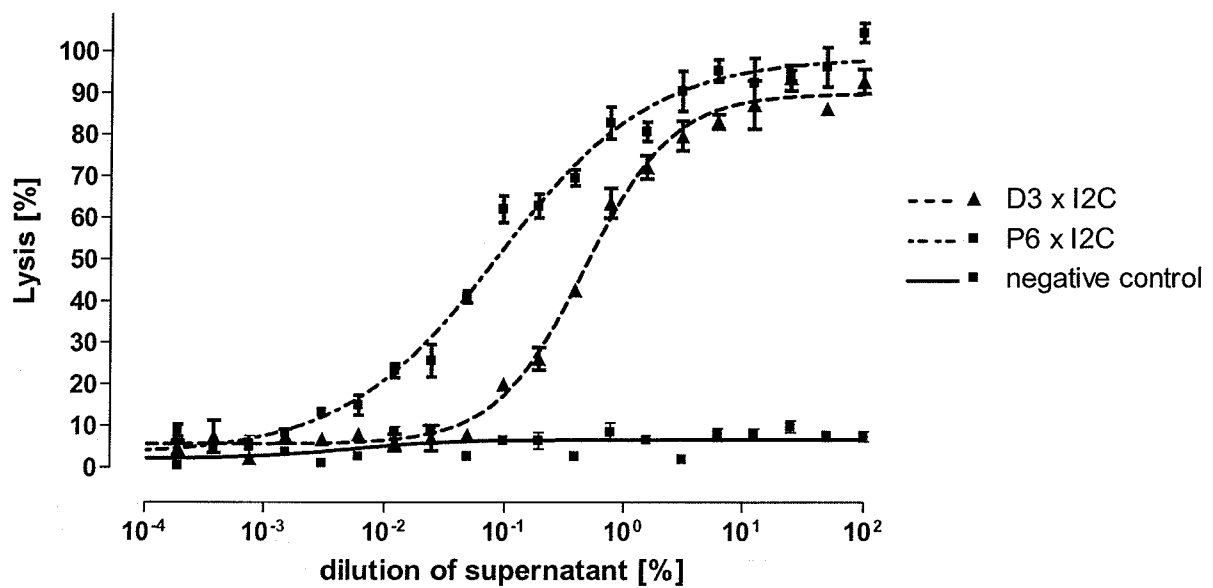


Figure 8

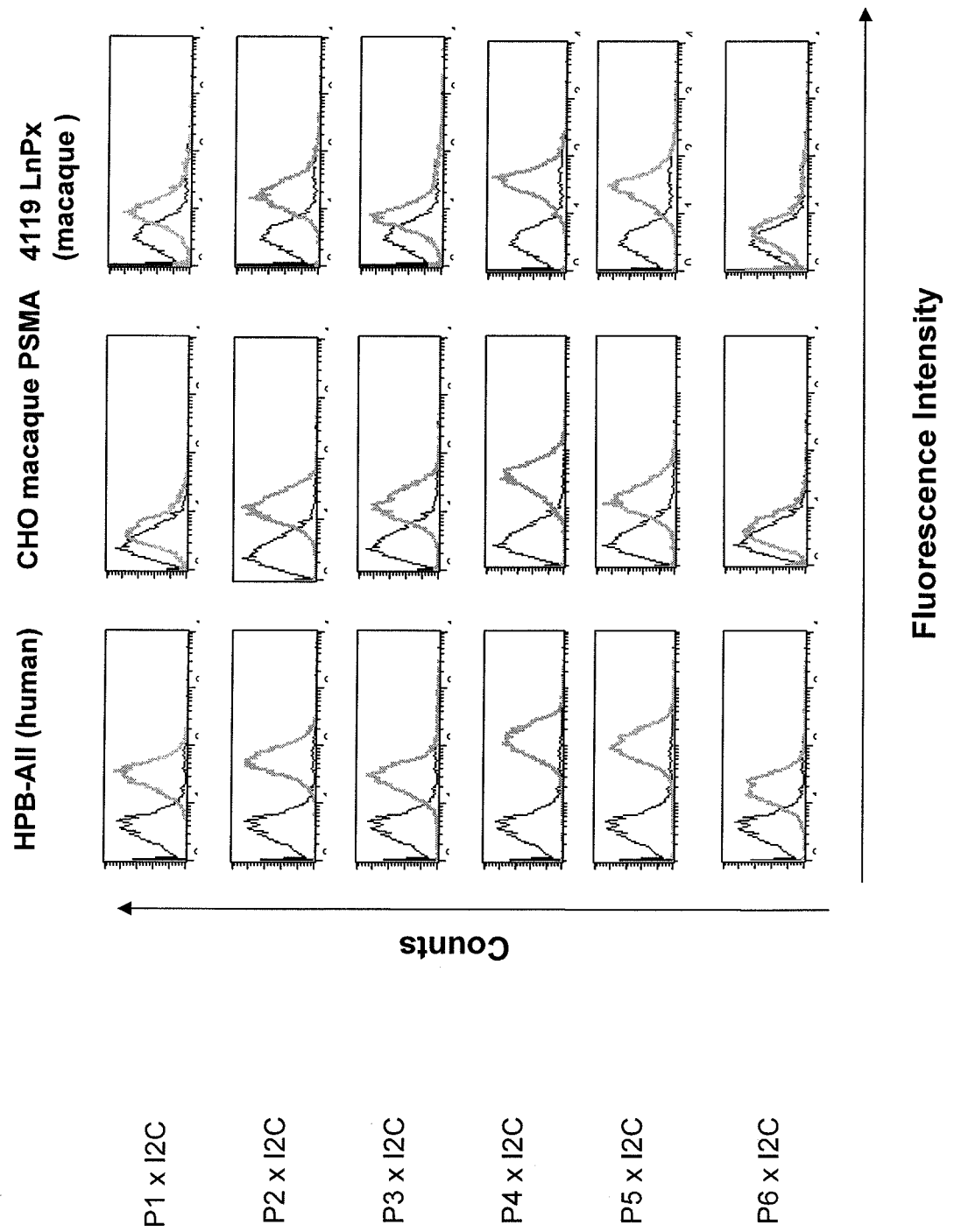
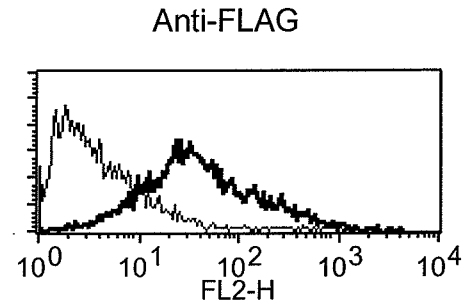


Figure 9

FAPalpha human	1	MKTWVKIIVFGVATSAVLALALVMCIVLRPSRVHNSENTMRALTTLKDLNGTFSYKTFFPNWISQGEYLHQSDNNIVLYN	80
FAPalpha murine	81	MKTWIKTVFGVTTLAALALVVICIVLRPSRVYKPEGNTKRALTTLKDLNGTFSYKTFFPNWISQGEYLHQSEDDNIVFY	160
FAPalpha human		IETGQSYIILSNRTMKSVNASNYGLSPDRQFVYLESDYSKLWRYSYTATYYIDLSNGEFVVRGNELPRPIQYLCWSPVGS	
FAPalpha-murine		IETRESYIILSNSMTMKSVNATDYGGLSPDRQFVYLESDYSKLWRYSYTATYYIDLQNGEFVVRGYELPRPIQYLCWSPVGS	160
FAPalpha human	161	KLAYVYQNNIYLKQRPDPFPFQITFNGRENKIFNGIPDWVYEEEMLATKYALWSPNGKFLAYAAEFNDTDIPVIAYSYYG	240
FAPalpha-murine		KLAYVYQNNIYLKQRPDPFPFQITTYTGRENRIENGIPDWVYEEEMLATKYALWSPDYGKFLAYVEFNDSDIPIIAYSYYG	240
FAPalpha human	241	DEQYPRITINIPYPKAGAKNPVVRIFIIDTTYPAVVGPEQEVVPVPAAMIASDDYFWSLWTWVTDTERVCLQWLKRQNVSVLSI	320
FAPalpha-murine		DGQYPRITINIPYPKAGAKNPVVRVFIVDTTYPHHVGPMEVVPVPEMIASSDDYFWSLWTWVSSSERVCLQWLKRQNVSVLSI	320
FAPalpha human	321	CDFREDWQTWDCEPKTQEHIEESRTGWAGGFVSTPVSFYDAISYYKIFSDKDGKYKHIHYIKDTVENAIQITSGKWEAINI	400
FAPalpha-murine		CDFREDWHAWECPKNQEHVEESRTGWAGGFVSTPAFSQDATSYYYKIFSDKDGKYKHIHYIKDTVENAIQITSGKWEAIYI	400
FAPalpha human	401	FRVTQDSLIFYSSNEFEFEYYPGRRNIYRISIGSYPPSKKCVTCHLRKERCQYYTASFSDYAKYYALVCYGPGLPISTLHDGR	480
FAPalpha-murine		FRVTQDSLIFYSSNEFEFGYPGRRNIYRISIGNSPPSKKCVTCHLRKERCQYYTASFSSYKAKYYALVCYGPGLPISTLHDGR	480
FAPalpha human	481	TDQEIKILEENKELENAKNIQLPKEIKKLEVDEITLWYKMIPLPPQFDRSKKYPLLIQVYGGPCSQSVRSVFAVNWISY	560
FAPalpha-murine		TDQEIQVLEENKELENSLRNIQLPKVEIKKLDGGLTFWYKMIPLPPQFDRSKKYPLLIQVYGGPCSQSVKSVFAVNWITTY	560
FAPalpha human	561	LASKEGMVIALVDGRGTAQGDGKLLYAVYRKLGVYEVEDQITAVRKFIEMGFIDEKRIAIWGSYGGYVSSLALASGTGL	640
FAPalpha-murine		LASKEGIVIALVDGRGTAQGDGKFLHAVYRKLGVYEVEDQILTAVRKFIEMGFIDEERIAIWGSYGGYVSSLALASGTGL	640
FAPalpha human	641	FKCGIAVAPVSSWEYYASVYTERFMGLPTKDDNLEHYKNSTVMARAEYFRNVLDYLLIHGTADDNVHFQNSAQIAKALVNA	720
FAPalpha-murine		FKCGIAVAPVSSWEYYASISYSERFMGLPTKDDNLEHYKNSTVMARAEYFRNVLDYLLIHGTADDNVHFQNSAQIAKALVNA	720
FAPalpha human	721	QVDFQAMWYSDQNHGLSGLSTNHLTYTHMTFLKQCFSLSD	760
FAPalpha-murine		QVDFQAMWYSDQNHGHSGRSQNHLYTHMTFLKQCFSLSD	760

CHO transfected with a mutated human
FAPA antigen with murine membrane-distal
epitopes



CHO transfected with
murine FAPA

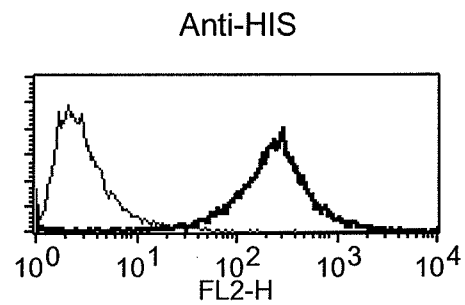


Figure 10

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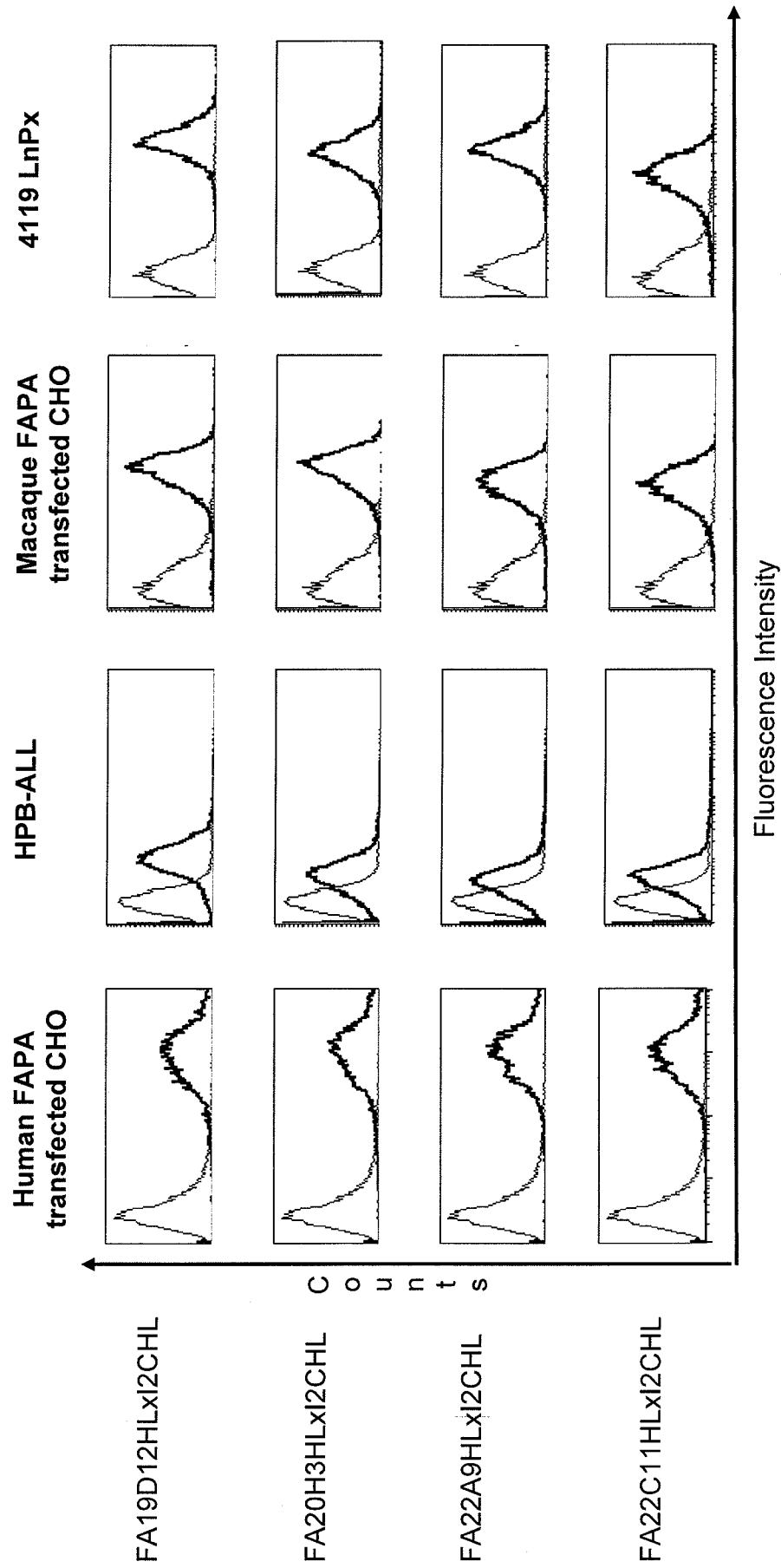


Figure 11a

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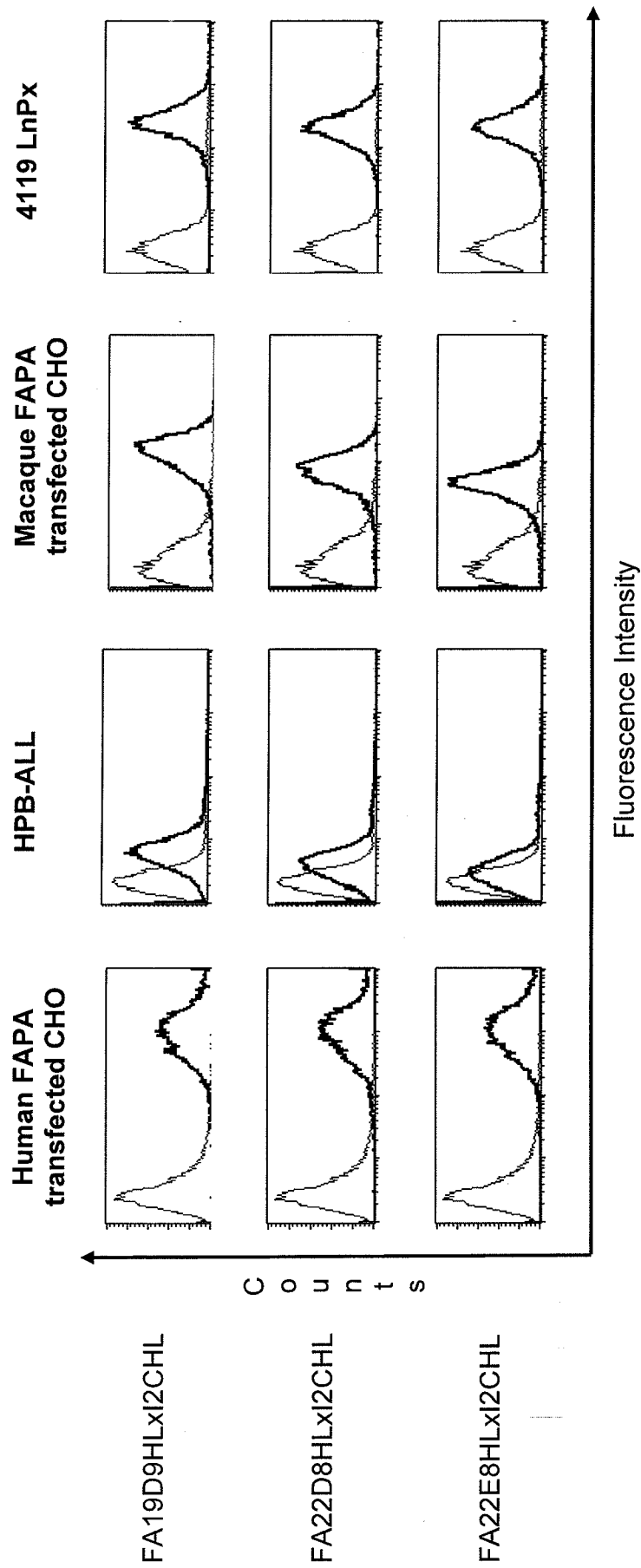
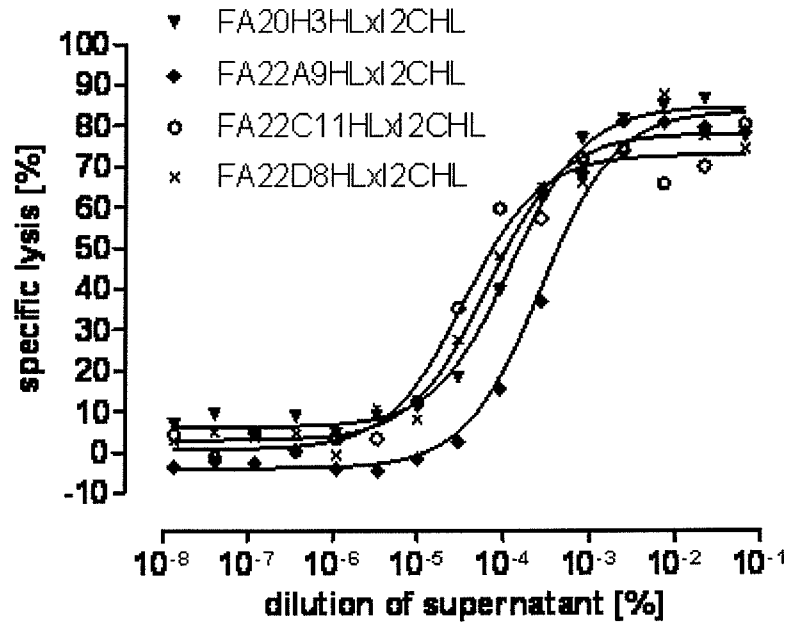


Figure 11b

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Effector T cells: stimulated human CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human FAPA
E:T ratio: 10:1



Effector T cells: stimulated human CD4/CD56 depleted human PBMC
Target cells: CHO transfected with human FAPA
E:T ratio: 10:1

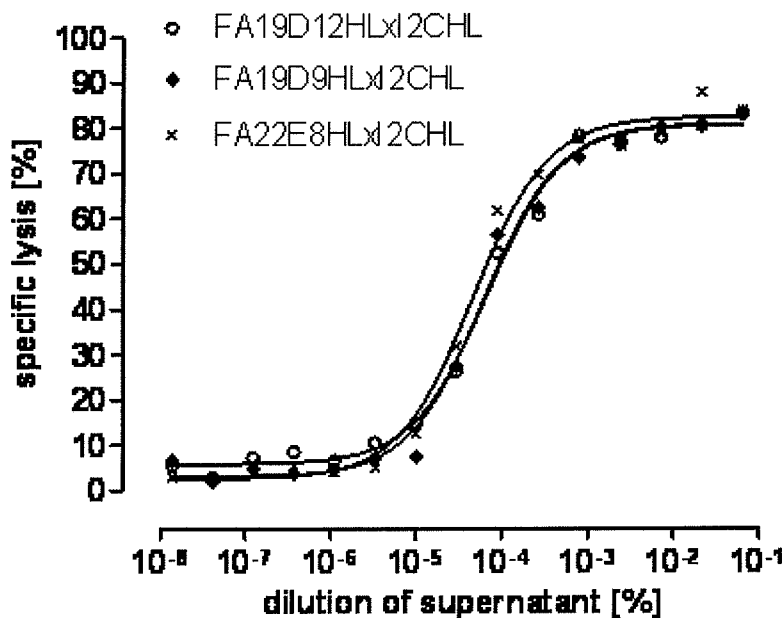
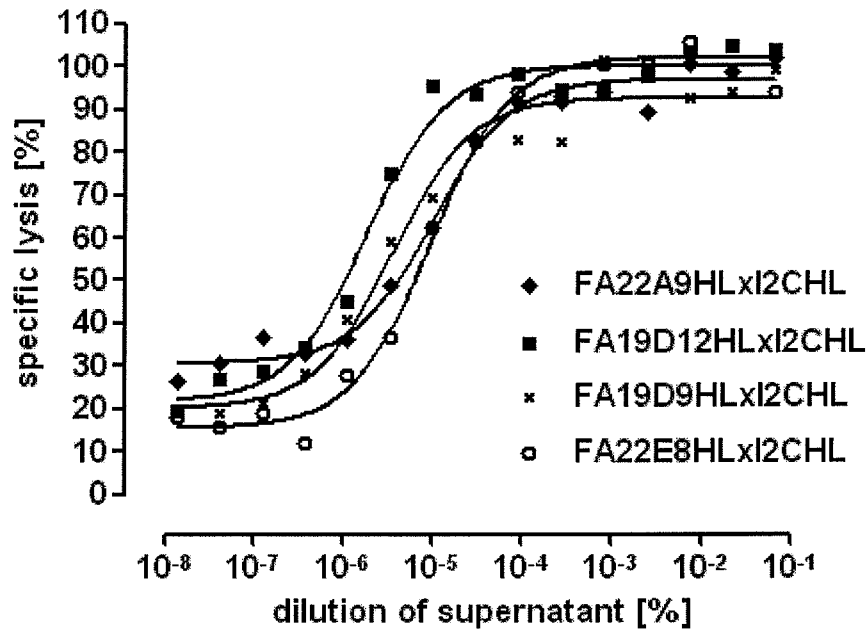


Figure 12a

Effector T cells: stimulated CD4/CD56 depleted human PBMC
 Target cells: CHO transfected with a mutated human FAPA antigen with
 murine membrane-distal epitopes
 E:T ratio: 10:1



Effector T cells: stimulated CD4/CD56 depleted human PBMC
 Target cells: CHO transfected with a mutated human FAPA antigen with
 murine membrane-distal epitopes
 E:T ratio: 10:1

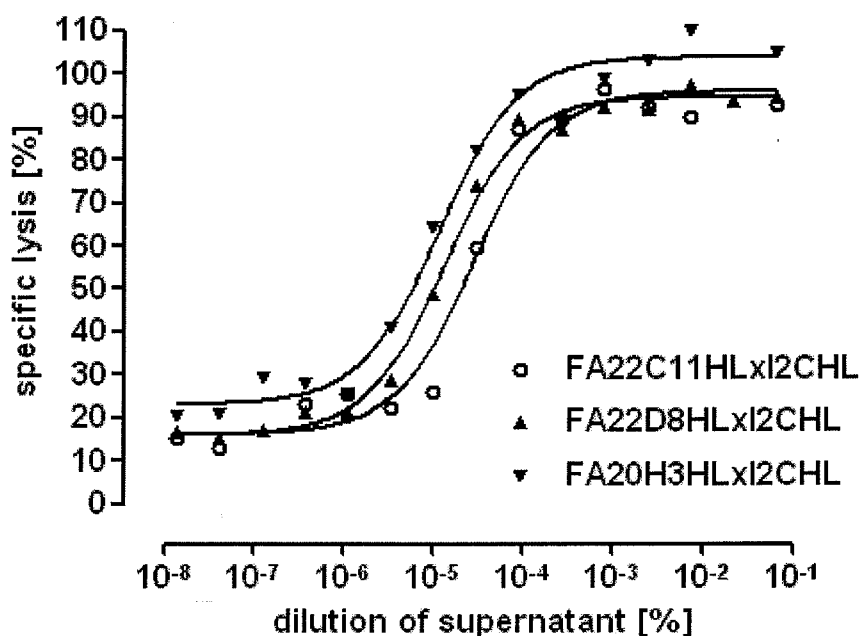
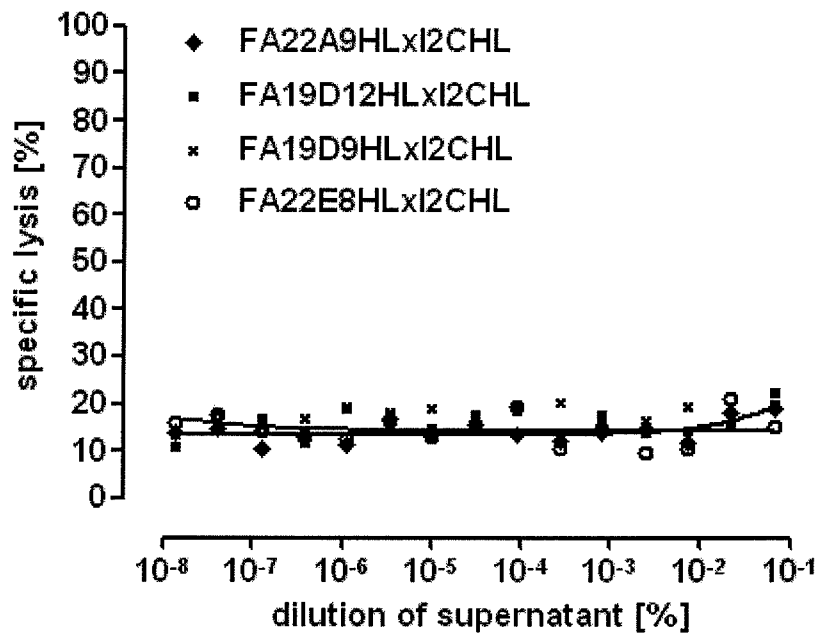


Figure 12b

Effector T cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with murine FAPA
E:T ratio: 10:1



Effector T cells: stimulated CD4/CD56 depleted human PBMC
Target cells: CHO transfected with murine FAPA
E:T ratio: 10:1

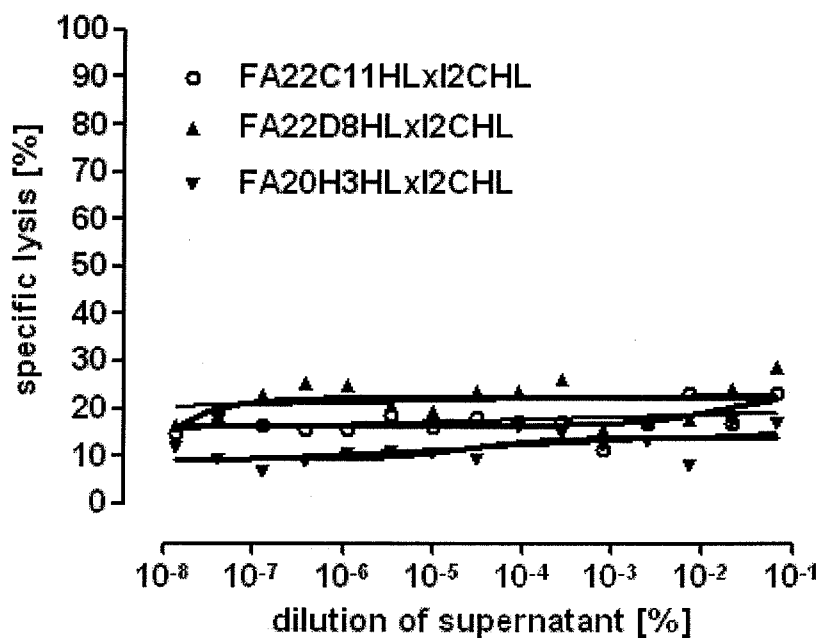


Figure 12c

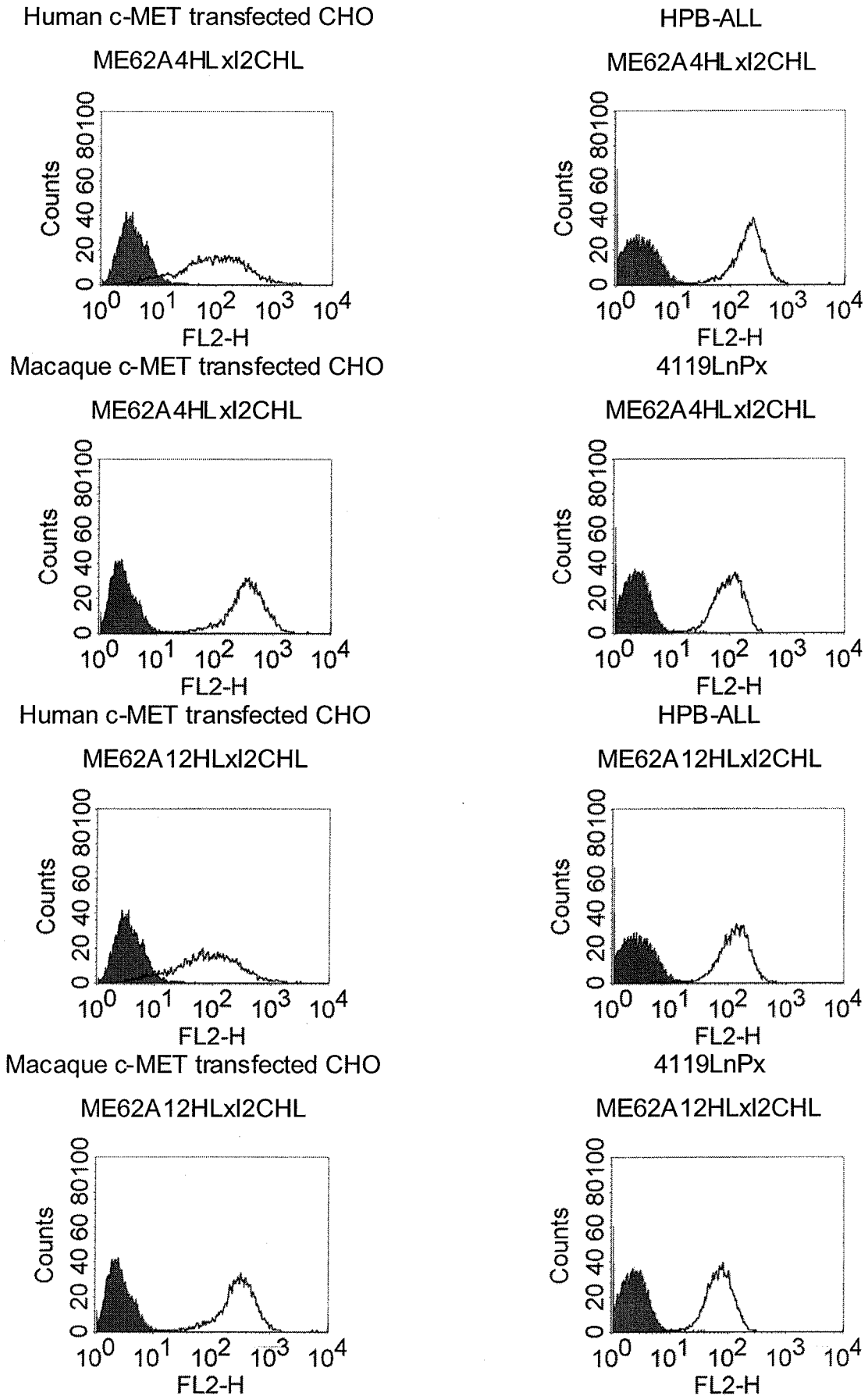


Figure 13a

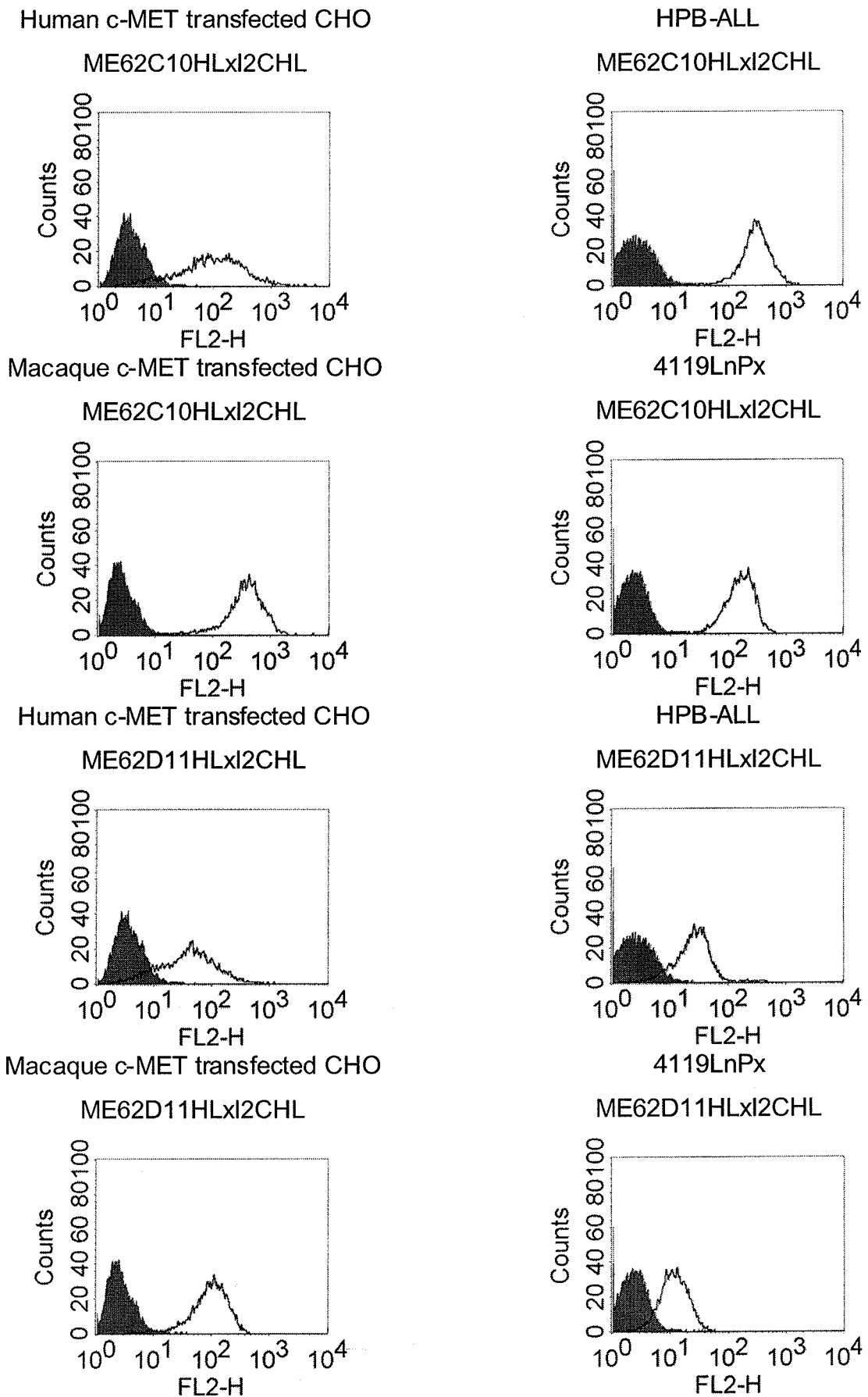


Figure 13b

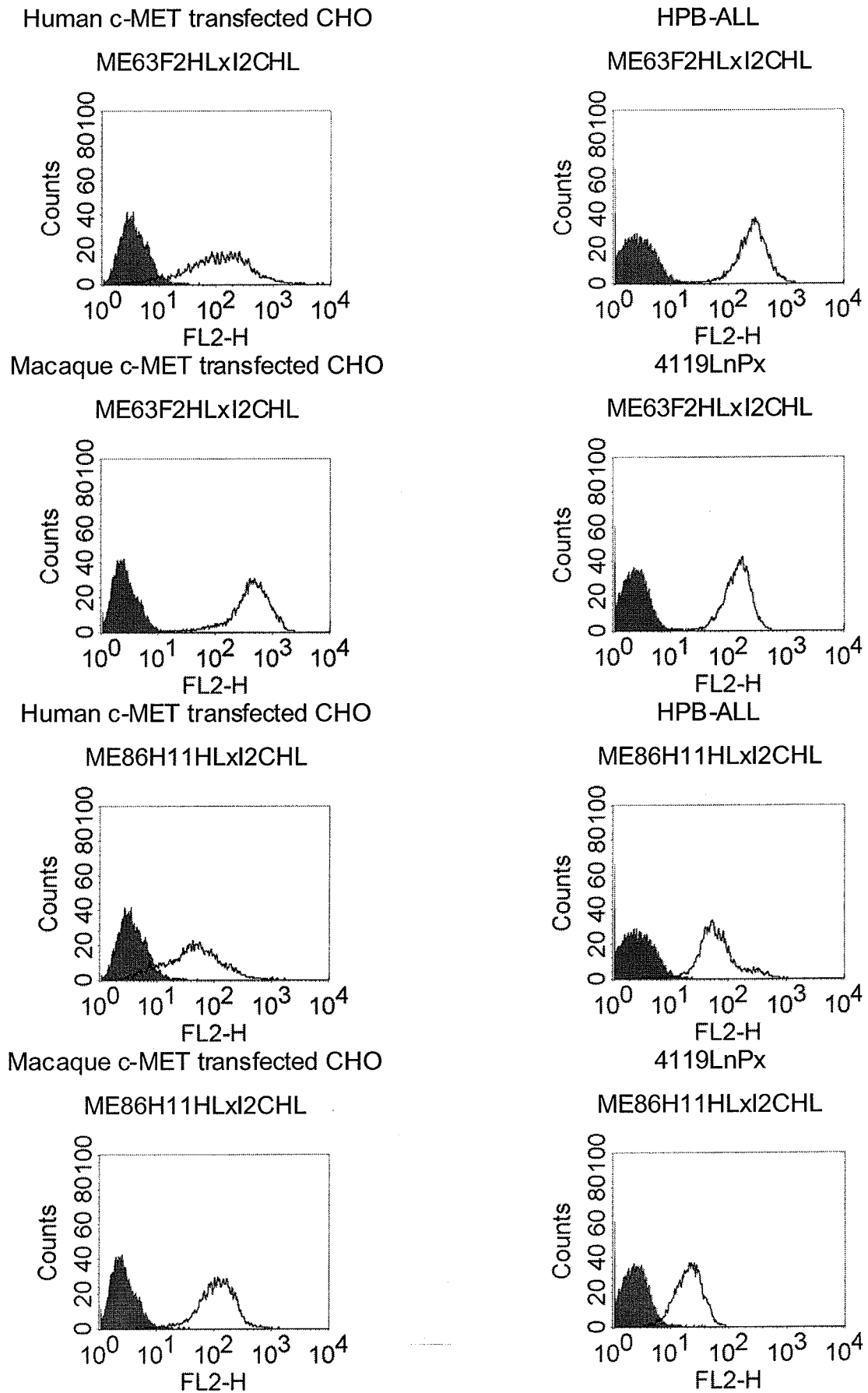


Figure 13c

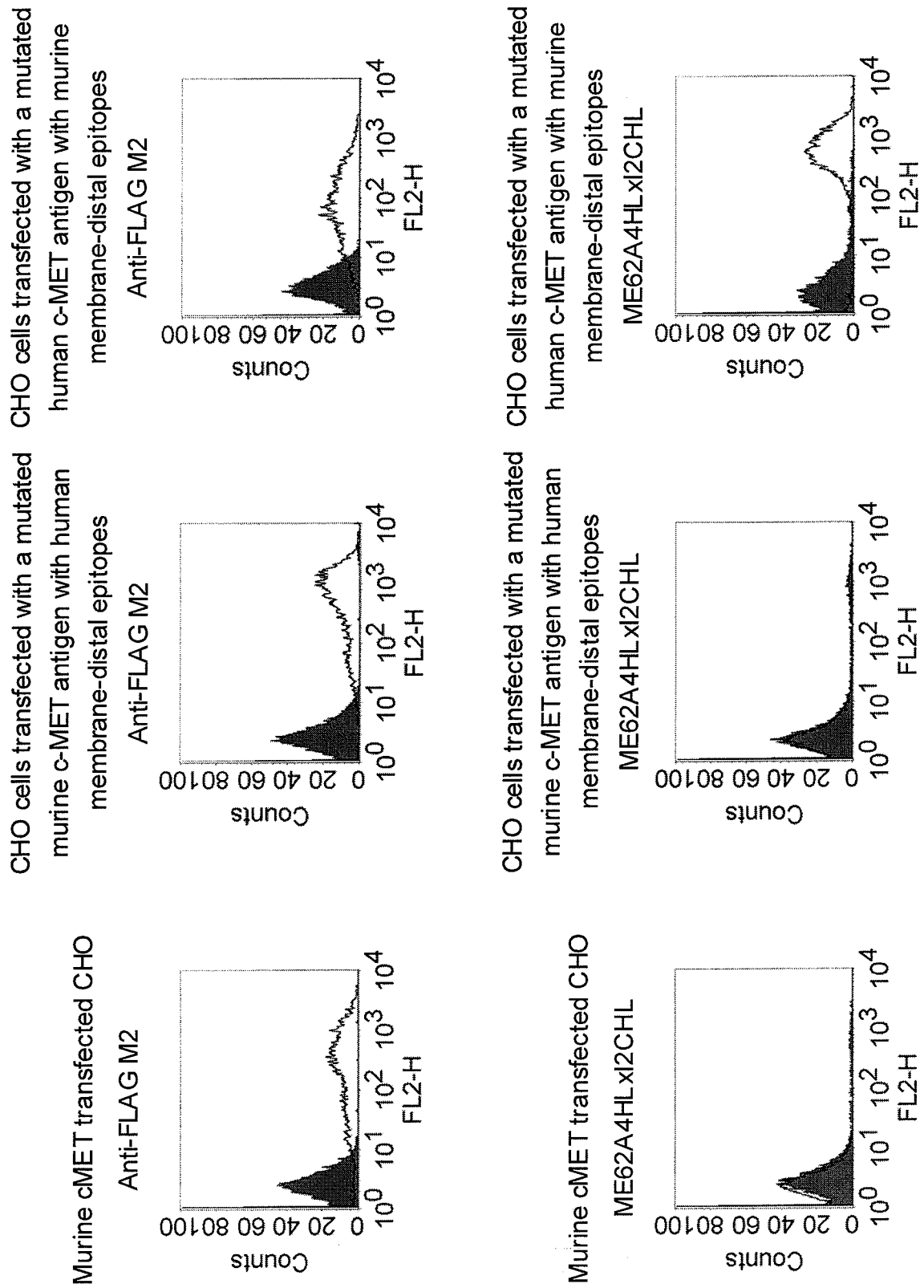


Figure 14a

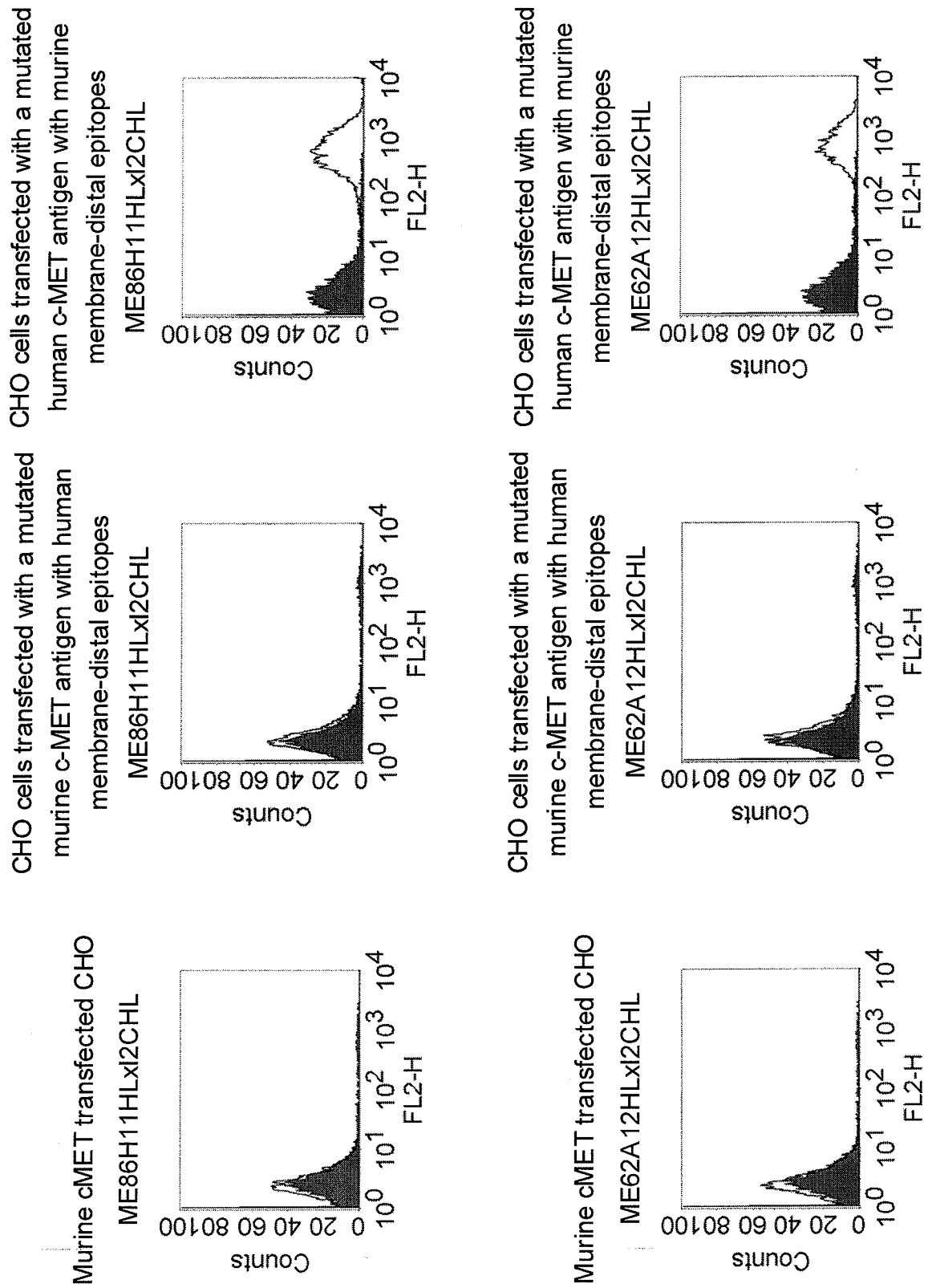


Figure 14b

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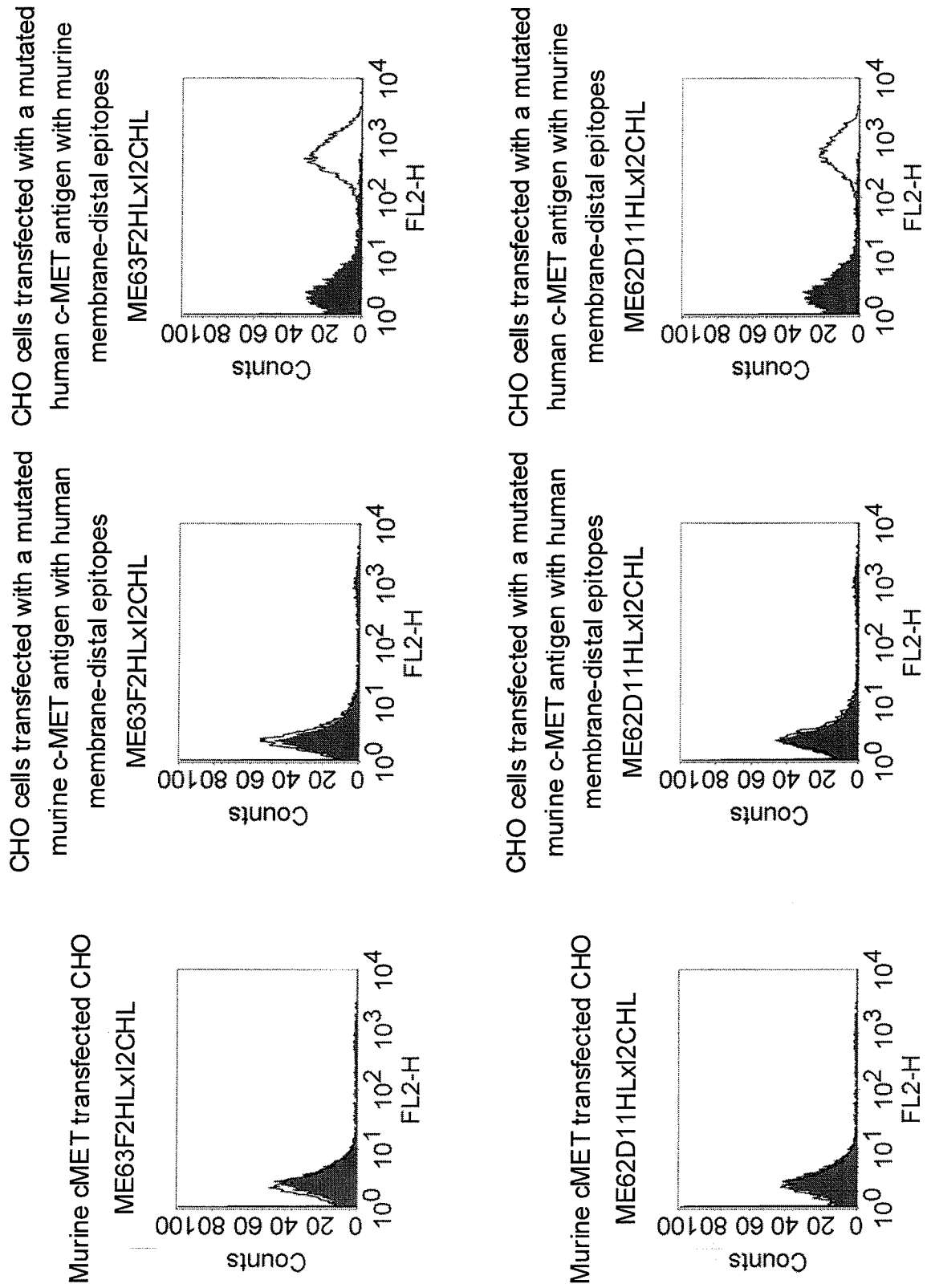


Figure 14c

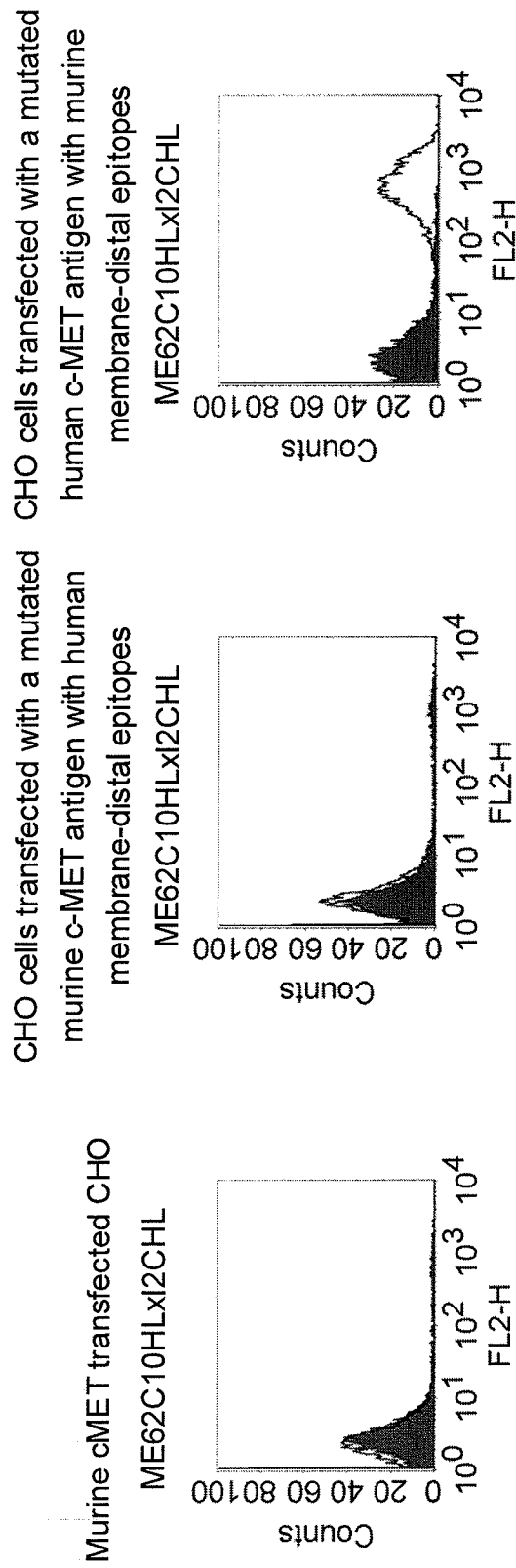
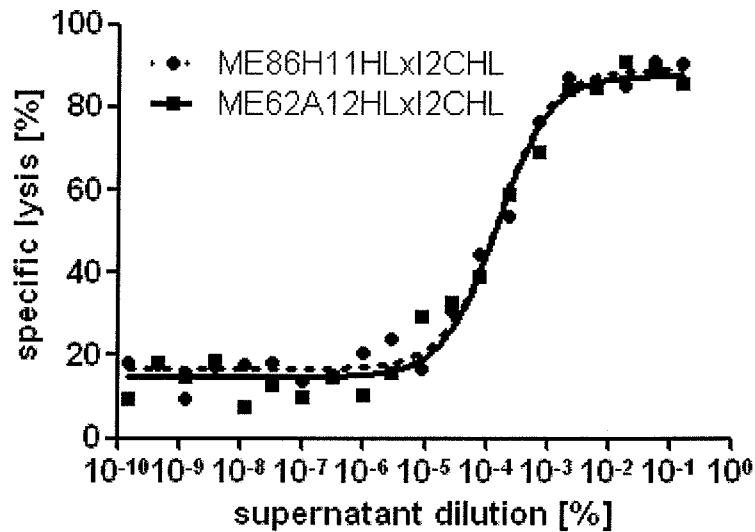


Figure 14d

Effector T cells: stimulated CD4/CD56 depleted human PBMC

Target cells: CHO tr<ansfected with human c-MET



Effector T cells: stimulated CD4/CD56 depleted human PBMC

Target cells: CHO transfected with human c-MET

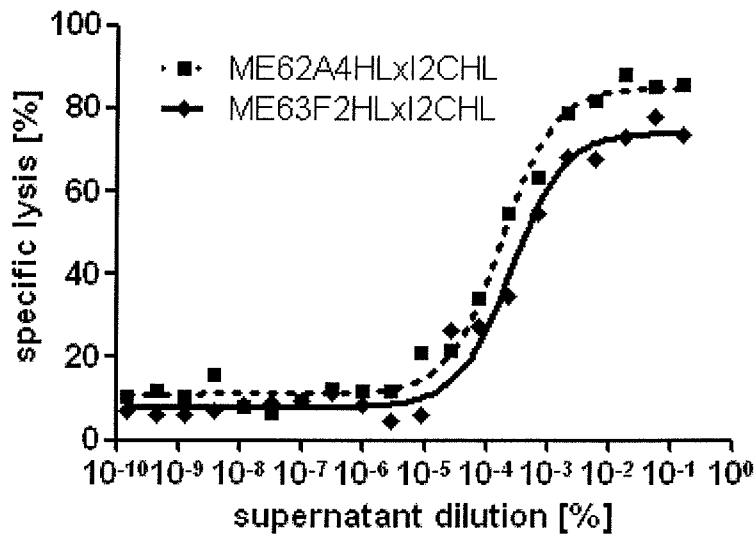
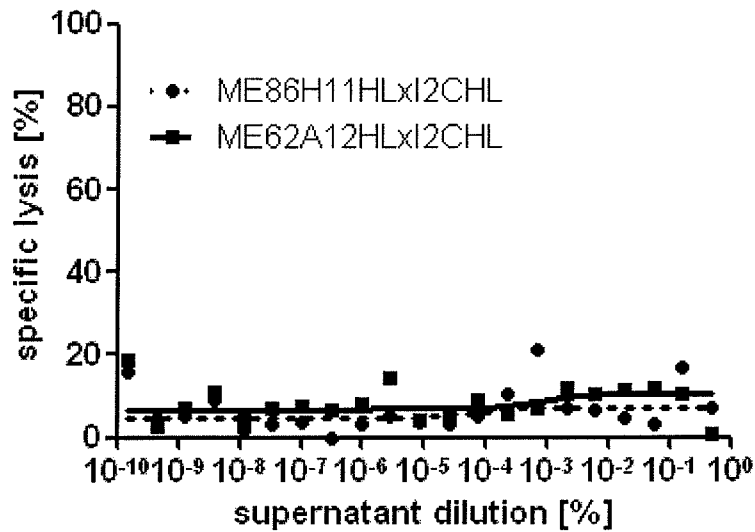


Figure 15a

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Effector T cells: stimulated CD4/CD56 depleted human PBMC

Target cells: CHO transfected with murine c-MET



Effector T cells: stimulated CD4/CD56 depleted human PBMC

Target cells: CHO transfected with murine c-MET

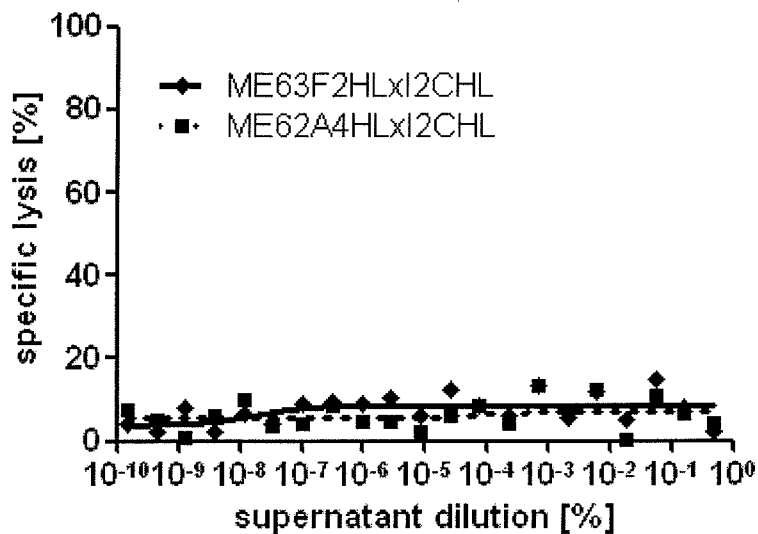
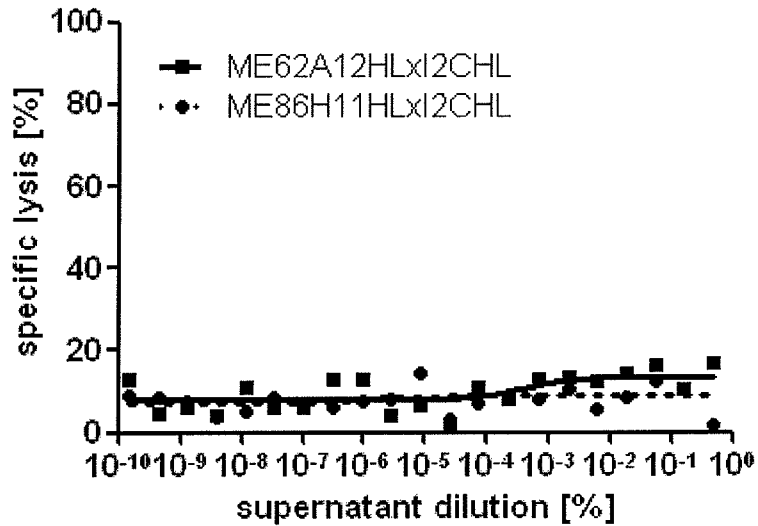


Figure 15b

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Effector T cells: stimulated CD4/CD56 depleted human PBMC

Target cells: CHO transfected with the mutated murine c-MET antigen
with human membrane-distal epitopes



Effector T cells: stimulated CD4/CD56 depleted human PBMC

Target cells: CHO transfected with the mutated murine c-MET antigen
with human membrane-distal epitopes

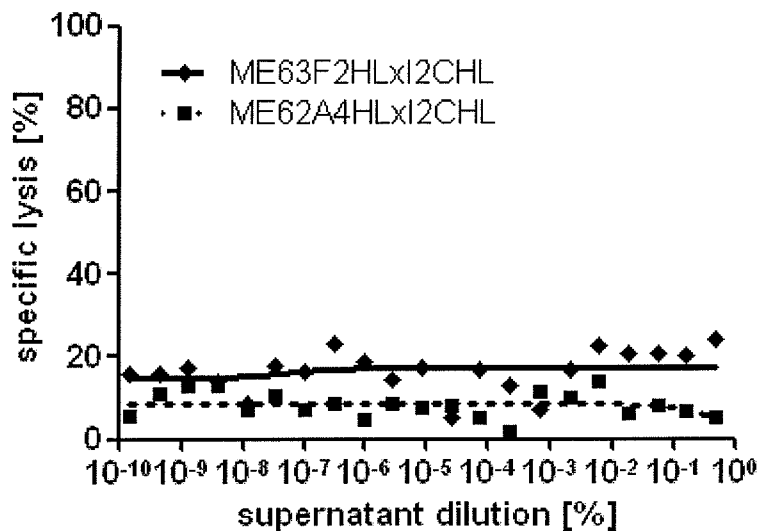


Figure 15c

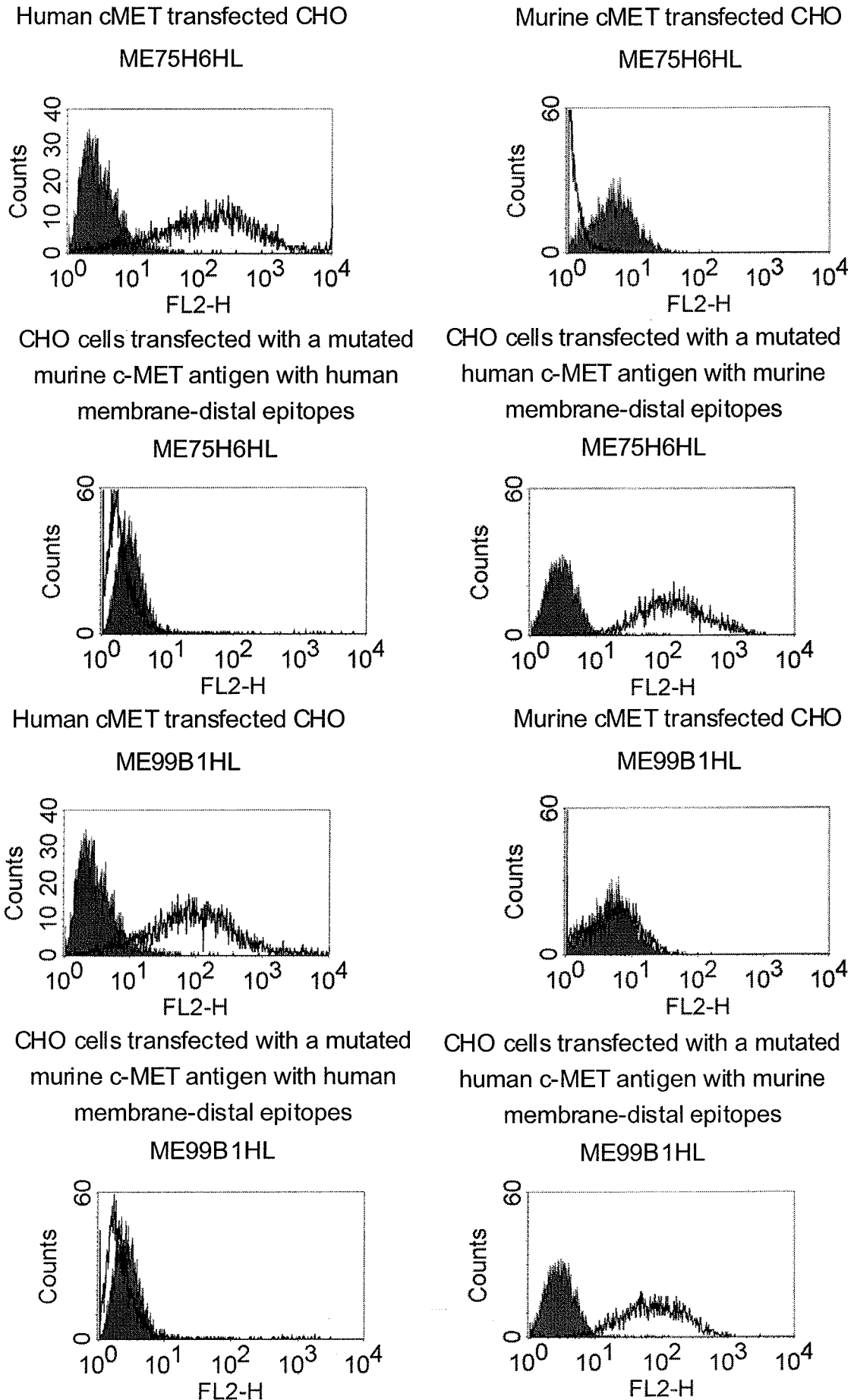
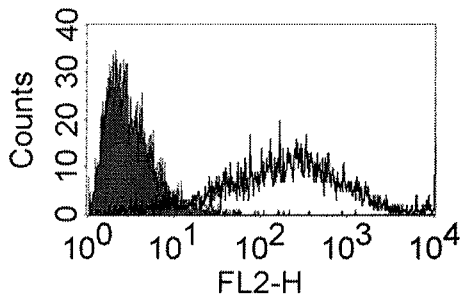
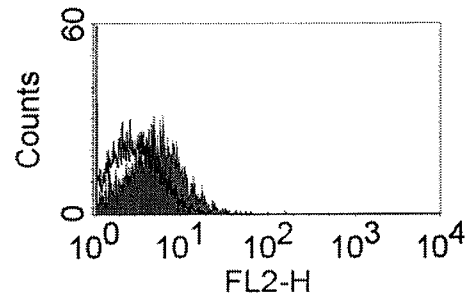


Figure 16a

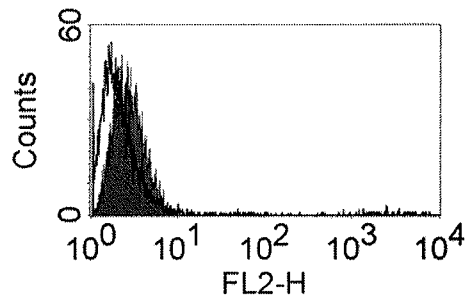
Human cMET transfected CHO
ME05B7HL



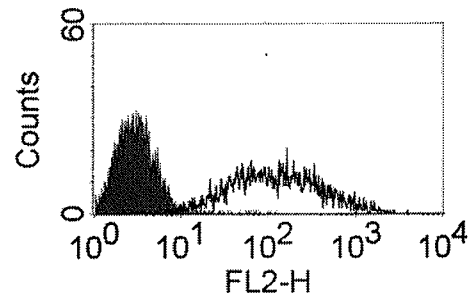
Murine cMET transfected CHO
ME05B7HL



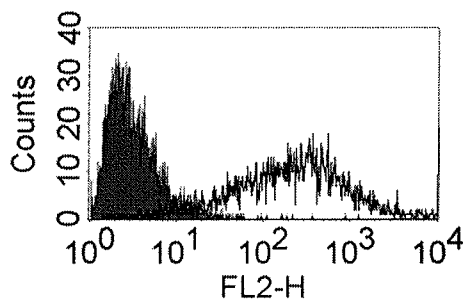
CHO cells transfected with a mutated
murine c-MET antigen with human
membrane-distal epitopes
ME05B7HL



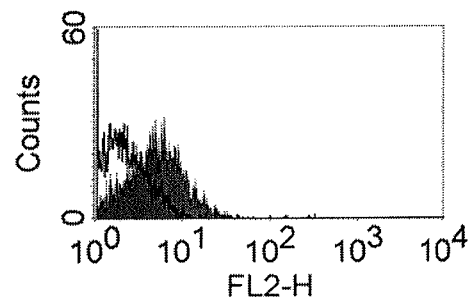
CHO cells transfected with a mutated
human c-MET antigen with murine
membrane-distal epitopes
ME05B7HL



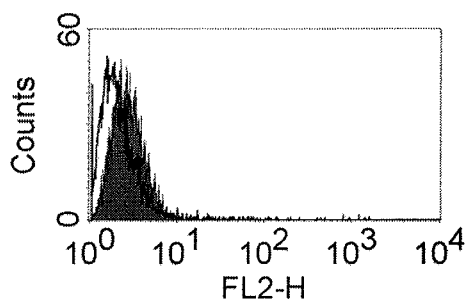
Human cMET transfected CHO
ME05F6HL



Murine cMET transfected CHO
ME05F6HL



CHO cells transfected with a mutated
murine c-MET antigen with human
membrane-distal epitopes
ME05F6HL



CHO cells transfected with a mutated
human c-MET antigen with murine
membrane-distal epitopes
ME05F6HL

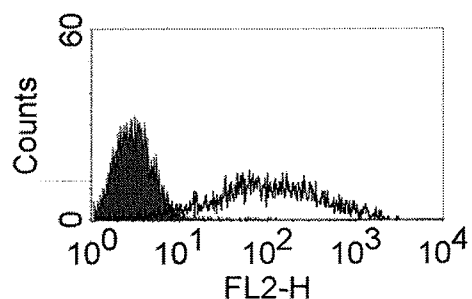


Figure 16b

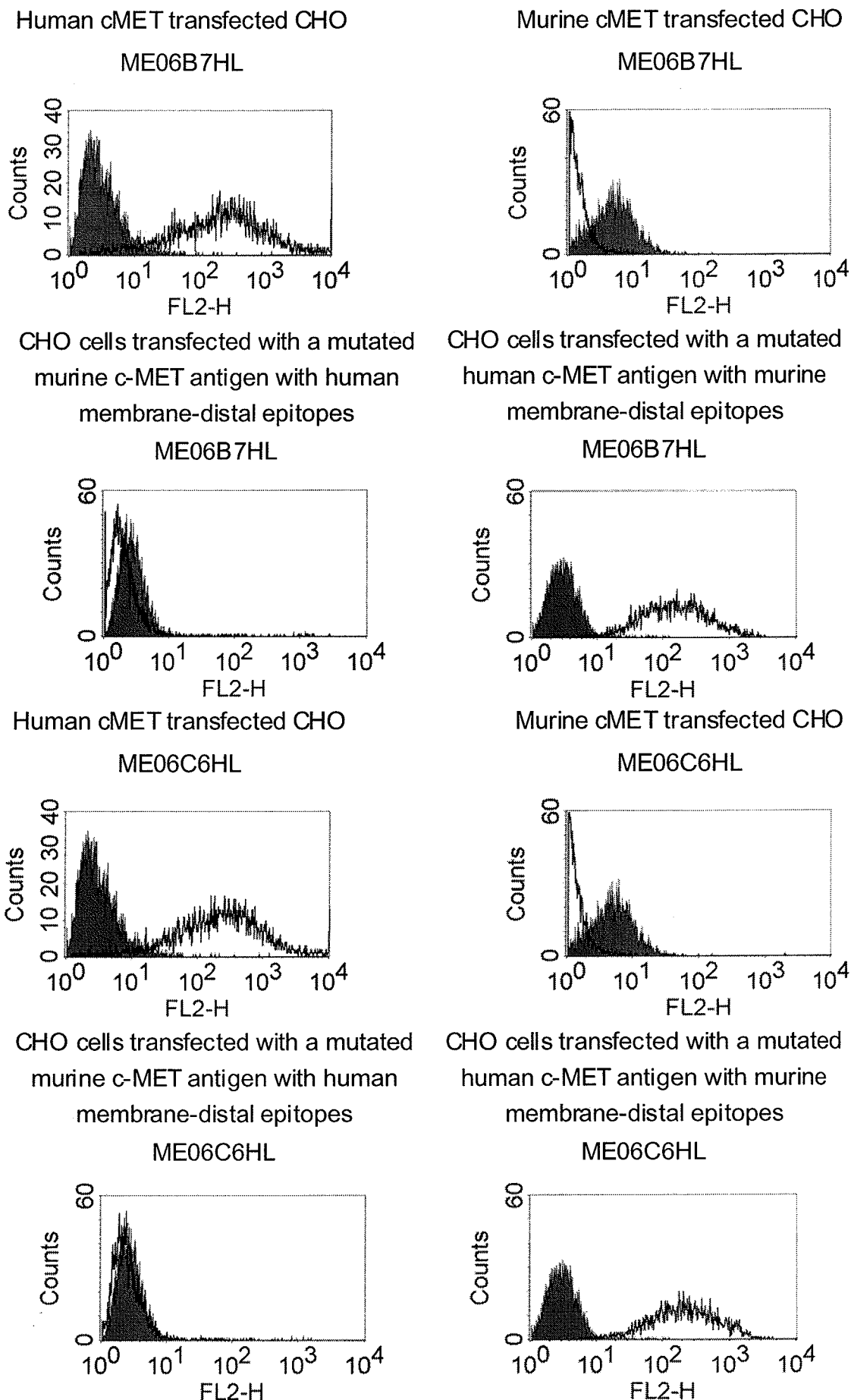


Figure 16c

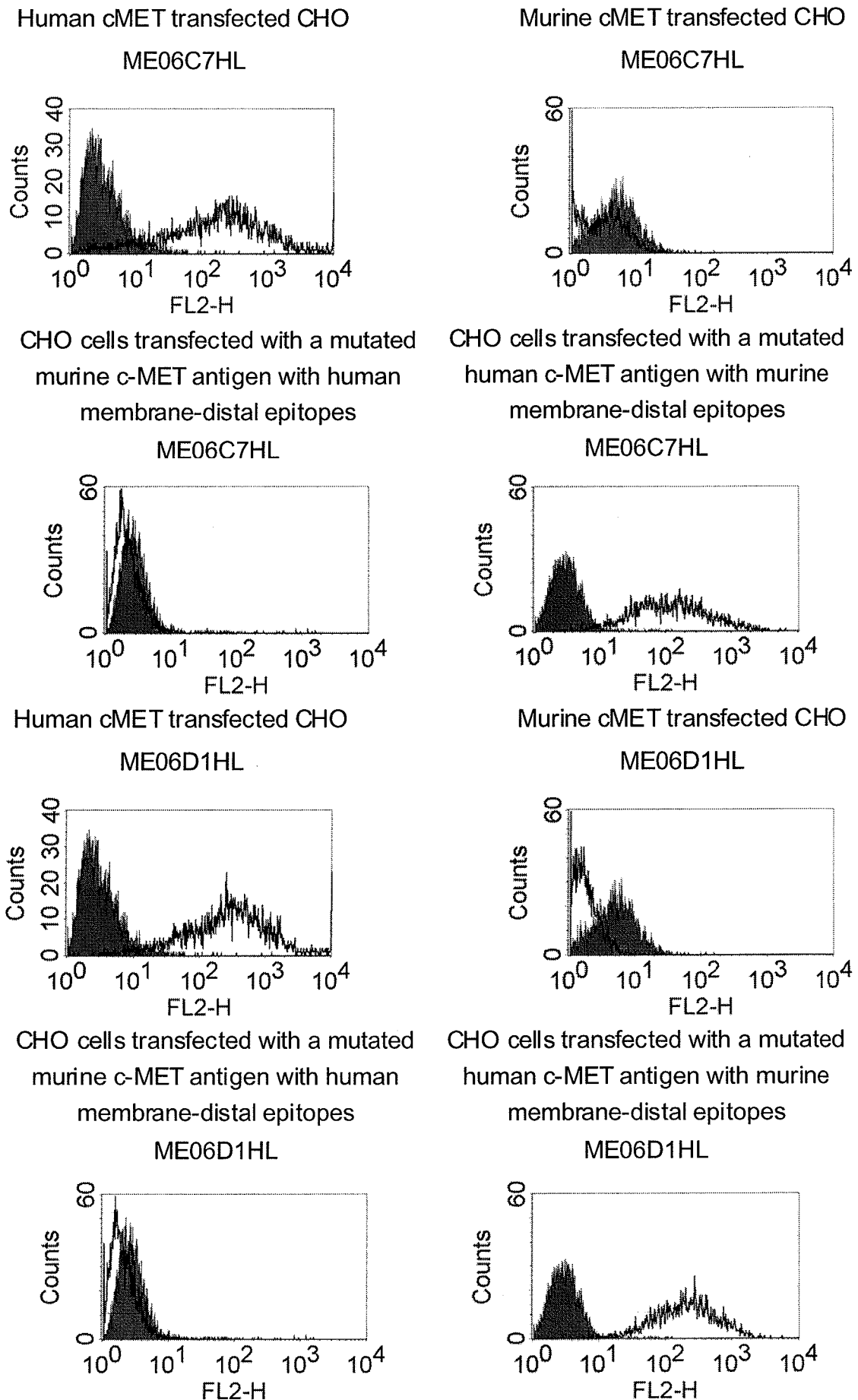
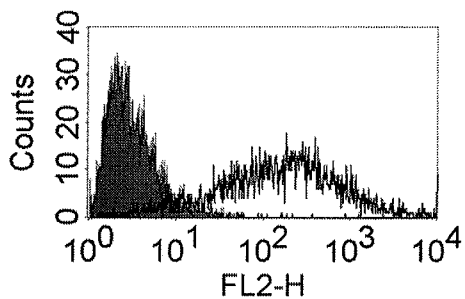
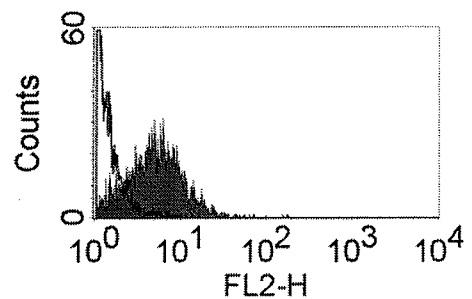


Figure 16d

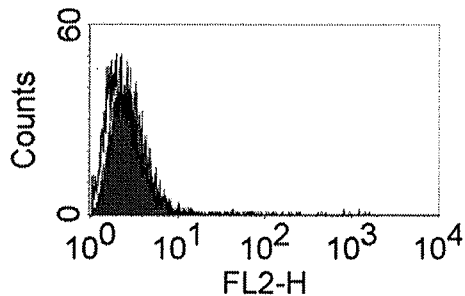
Human cMET transfected CHO
ME06D2HL



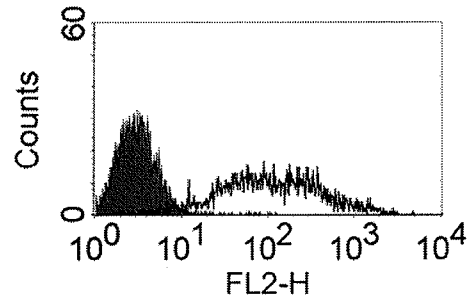
Murine cMET transfected CHO
ME06D2HL



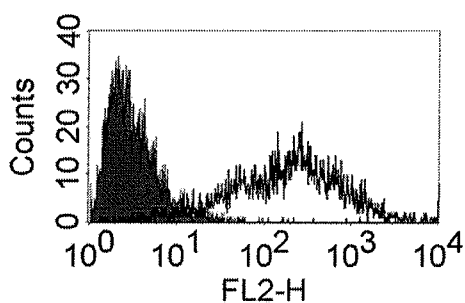
CHO cells transfected with a mutated
murine c-MET antigen with human
membrane-distal epitopes
ME06D2HL



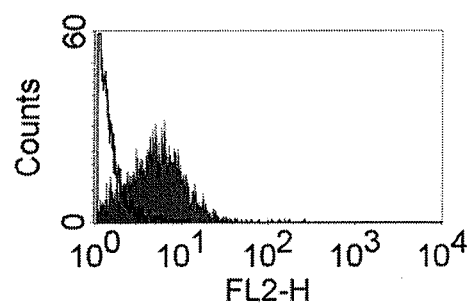
CHO cells transfected with a mutated
human c-MET antigen with murine
membrane-distal epitopes
ME06D2HL



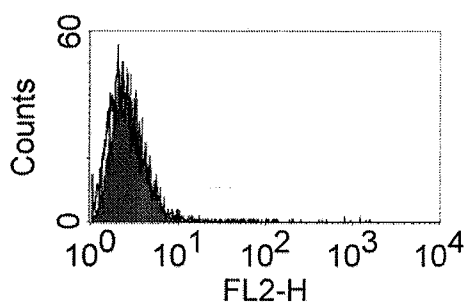
Human cMET transfected CHO
ME06E10HL



Murine cMET transfected CHO
ME06E10HL



CHO cells transfected with a mutated
murine c-MET antigen with human
membrane-distal epitopes
ME06E10HL



CHO cells transfected with a mutated
human c-MET antigen with murine
membrane-distal epitopes
ME06E10HL

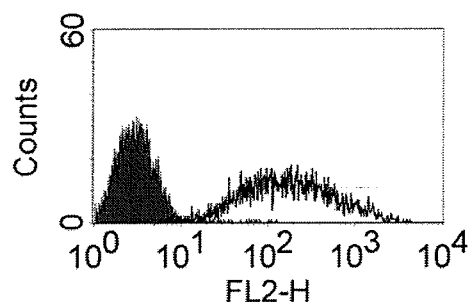
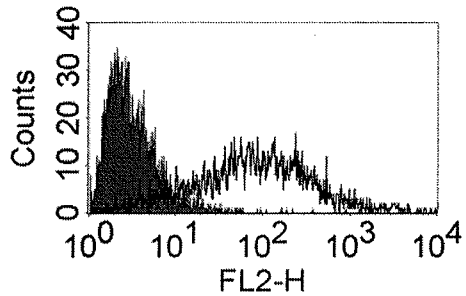
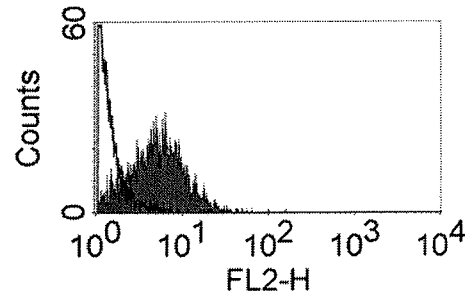


Figure 16e

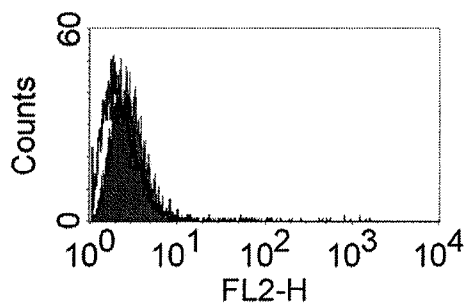
Human cMET transfected CHO
ME06F2HL



Murine cMET transfected CHO
ME06F2HL



CHO cells transfected with a mutated
murine c-MET antigen with human
membrane-distal epitopes
ME06F2HL



CHO cells transfected with a mutated
human c-MET antigen with murine
membrane-distal epitopes
ME06F2HL

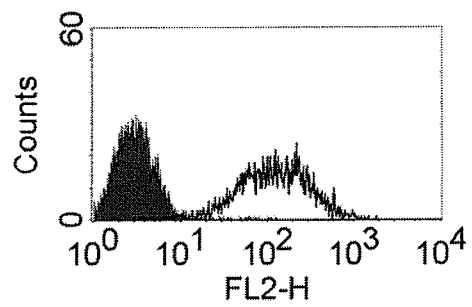
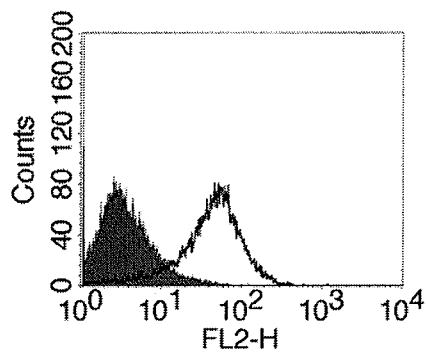


Figure 16f

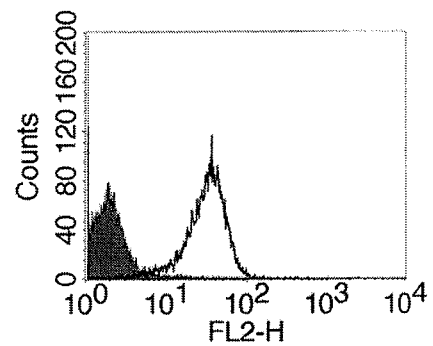
Human IGF-1R transfected CHO

IGF1R12HLxI2CHL



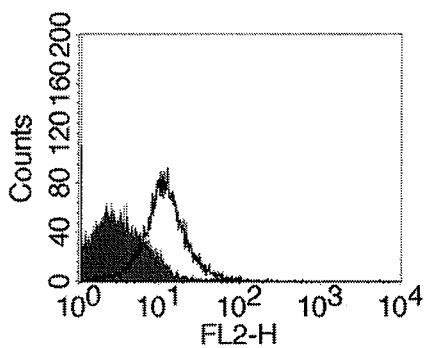
HPB-ALL

IGF1R12HLxI2CHL



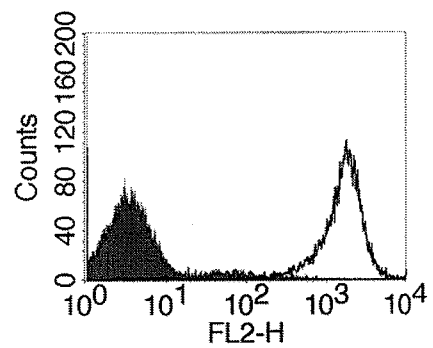
Macaque IGF-1R transfected CHO

IGF1R12HLxI2CHL



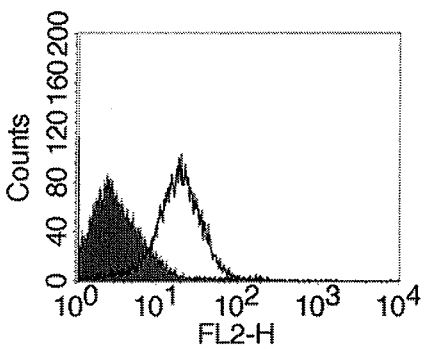
4119LnPx

IGF1R12HLxI2CHL



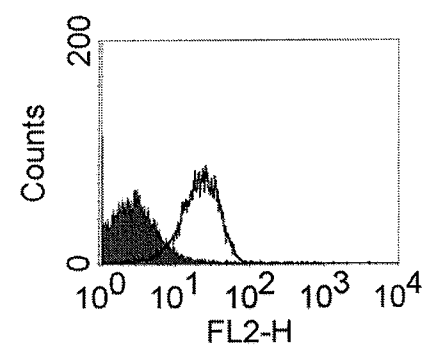
Human IGF-1R transfected CHO

IGF1R24HLxI2CHL



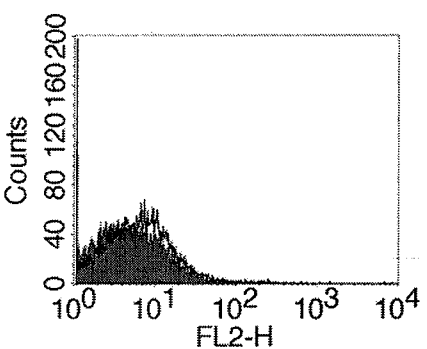
HPB-ALL

IGF1R24HLxI2CHL



Macaque IGF-1R transfected CHO

IGF1R24HLxI2CHL



4119LnPx

IGF1R24HLxI2CHL

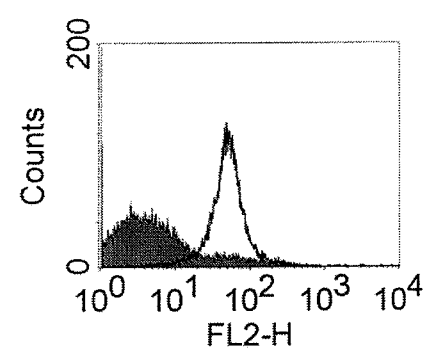


Figure 17

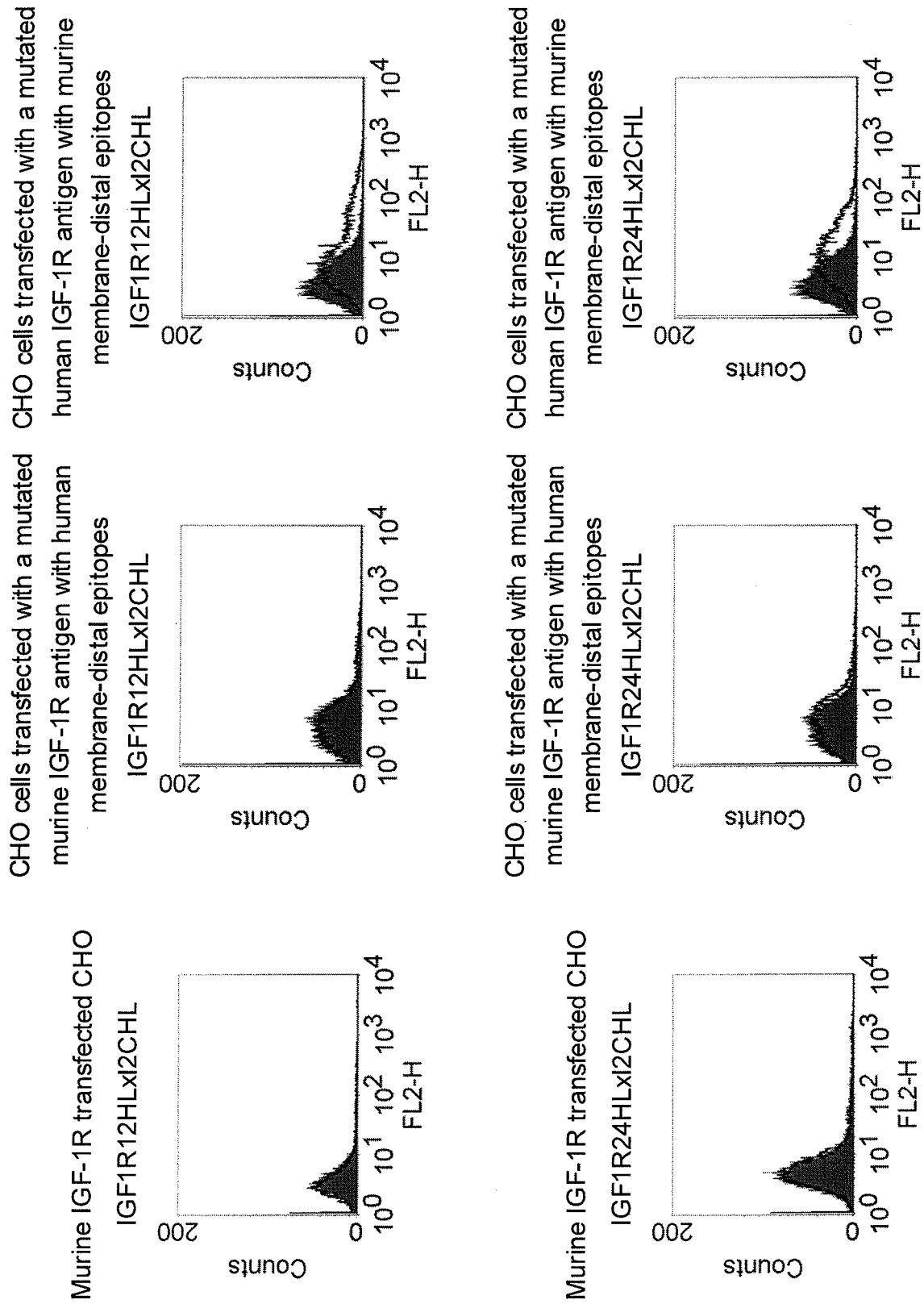


Figure 18

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Effector T cells: stimulated CD4/CD56 depleted human PBMC

Target cells: CHO transfected with human IGF-1R

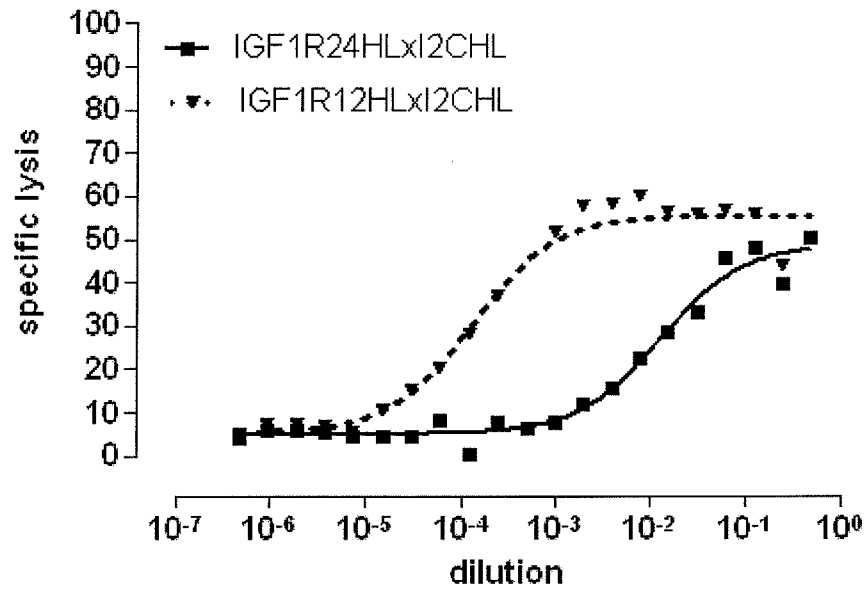
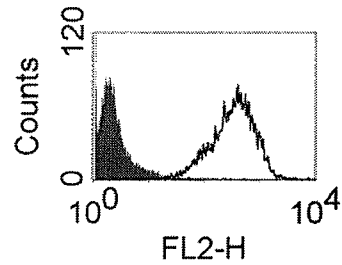
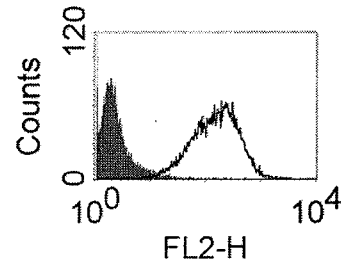


Figure 19

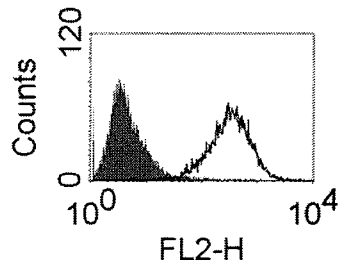
huPSMArat140-169 in CHO
PSMA-P7 HL x I2C HL



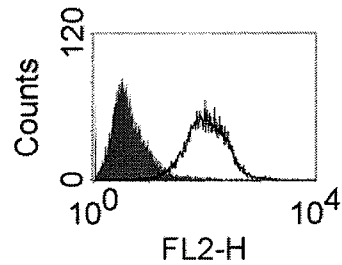
huPSMArat140-169 in CHO
PSMA-D4 HL x I2C HL



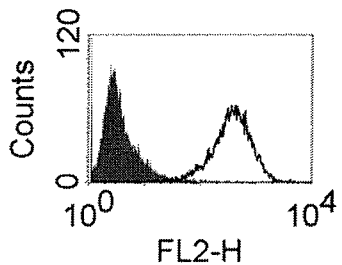
huPSMArat281-284 in CHO
PSMA-P7 HL x I2C HL



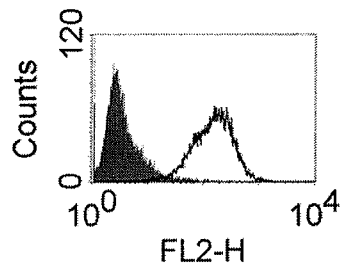
huPSMArat281-284 in CHO
PSMA-D4 HL x I2C HL



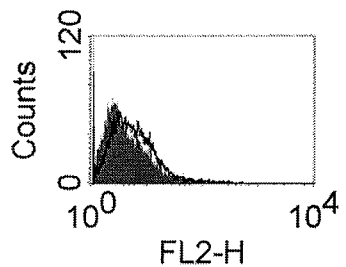
huPSMArat300-344 in CHO
PSMA-P7 HL x I2C HL



huPSMArat300-344 in CHO
PSMA-D4 HL x I2C HL



huPSMArat598-617 in CHO
PSMA-P7 HL x I2C HL



huPSMArat598-617 in CHO
PSMA-D4 HL x I2C HL

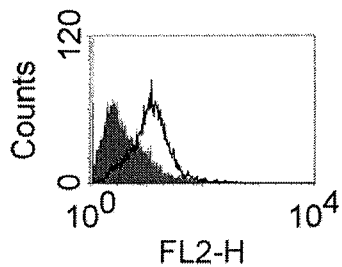
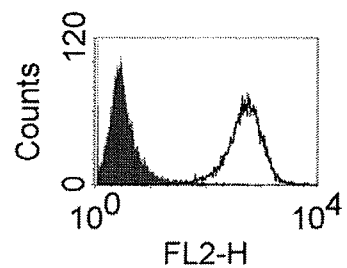


Figure 20a

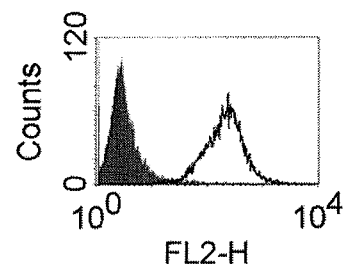
huPSMArat683-690 in CHO

PSMA-P7 HL x I2C HL



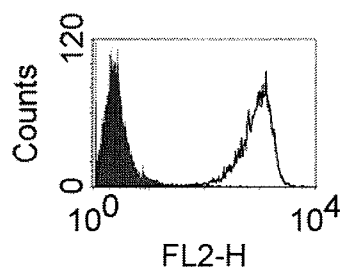
huPSMArat683-690 in CHO

PSMA-D4 HL x I2C HL



huPSMArat716-750 in CHO

PSMA-P7 HL x I2C HL



huPSMArat716-750 in CHO

PSMA-D4 HL x I2C HL

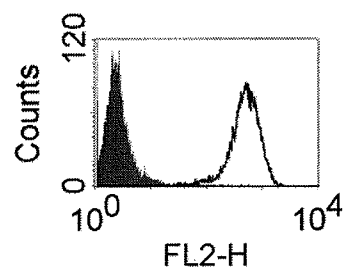


Figure 20b

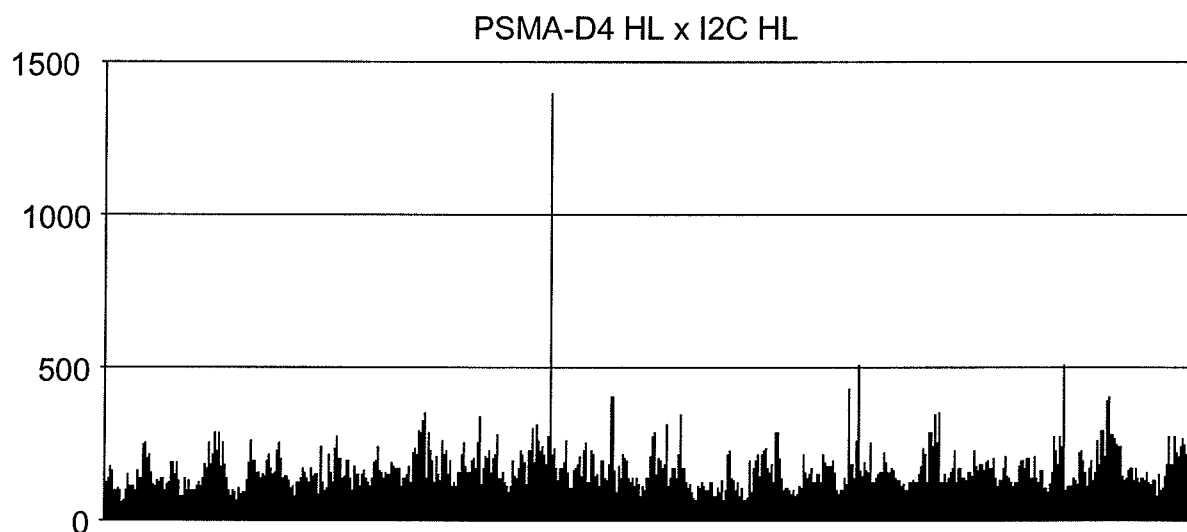


Figure 21

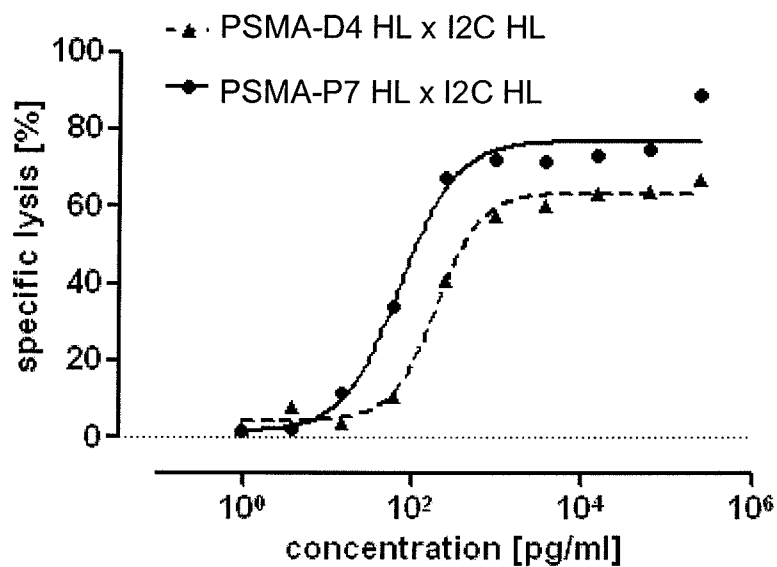


Figure 22