

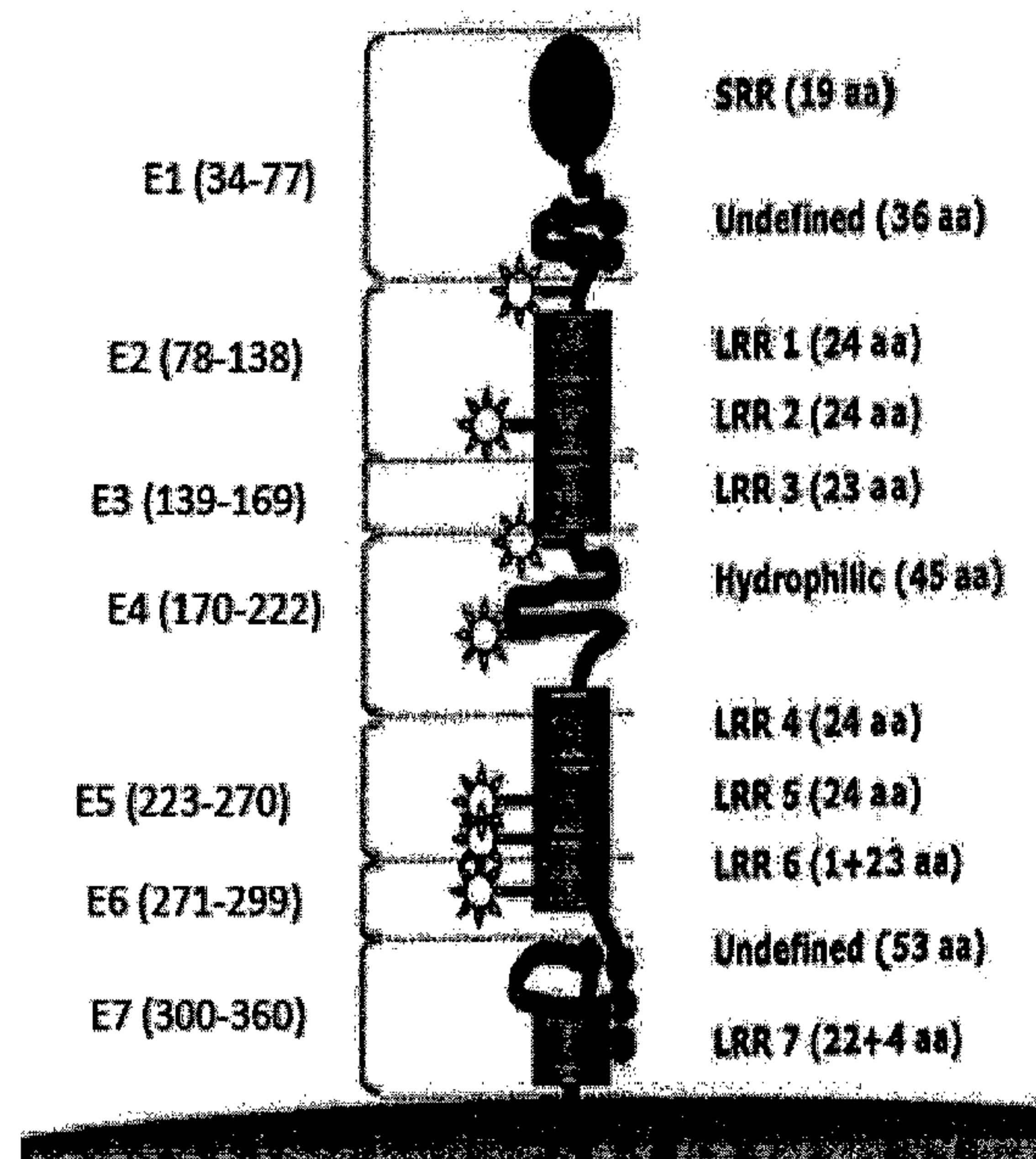


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(54) Title: BISPECIFIC BINDING MOLECULES FOR 5T4 AND CD3

Figure 2



(57) **Abrégé/Abstract:**

The present invention provides a bispecific binding molecule comprising a first and a second binding domain wherein the first binding domain is capable of binding to the epitope cluster 4 of T4 and the second binding domain is capable of binding to the CD3 receptor complex on T cells. Moreover, the invention provides a nucleic acid sequence encoding the bispecific binding molecule, a vector comprising said nucleic acid sequence and a host cell transformed or transfected with said vector. Furthermore, the invention provides a process for the production of the bispecific binding molecule of the invention, a medical use of said bispecific binding molecule and a kit comprising said bispecific binding molecule.

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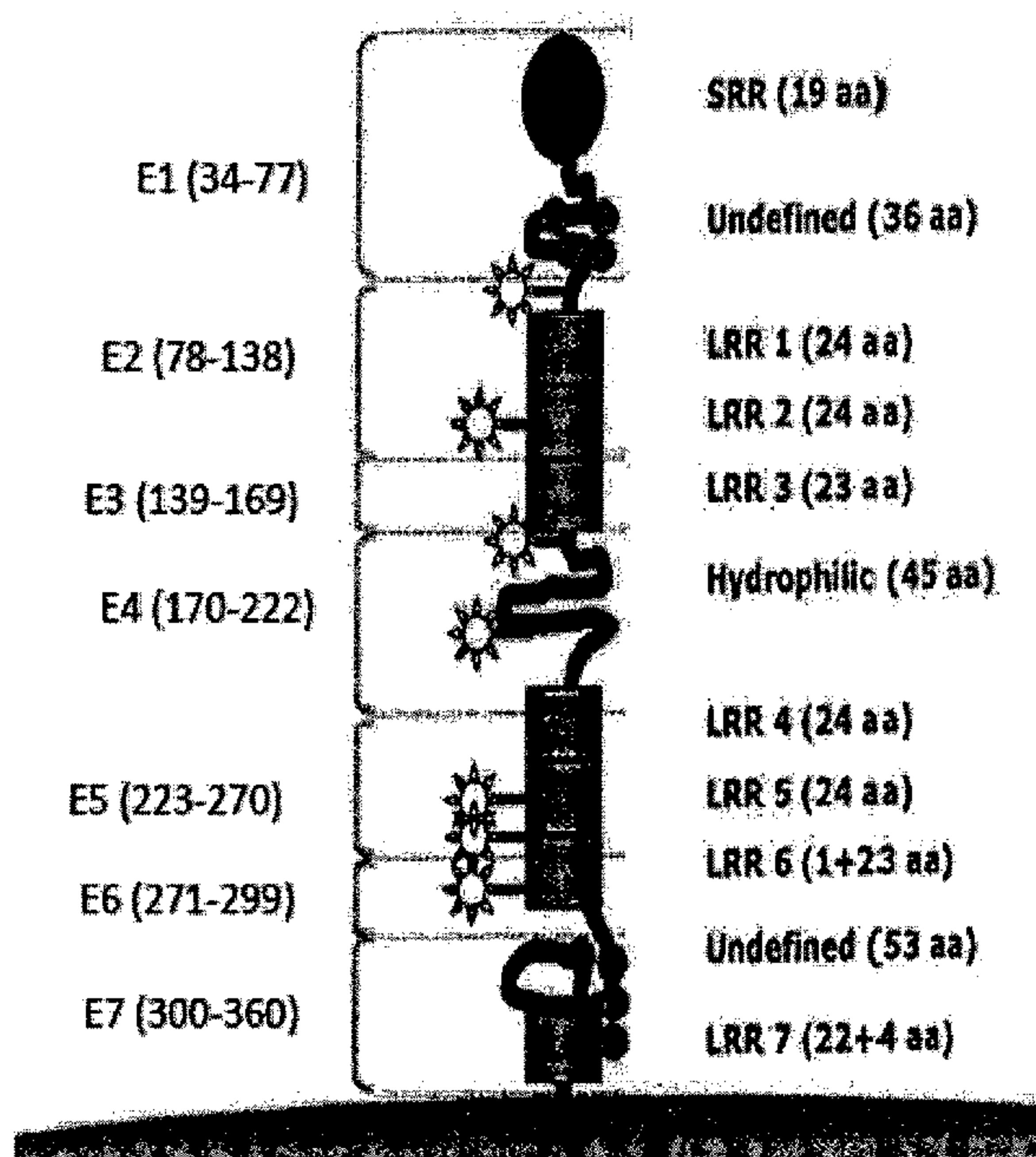
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Bispecific binding molecules for 5T4 and CD3

5 **[0001]** . The present invention relates to a bispecific binding molecule comprising a first and a second binding domain wherein the first binding domain is capable of binding to the epitope cluster 4 of 5T4 and the second binding domain is capable of binding to the CD3 receptor complex on T cells. Moreover, the invention relates to a nucleic acid sequence encoding the bispecific binding molecule, a vector comprising said nucleic acid sequence
10 and a host cell transformed or transfected with said vector. Furthermore, the invention relates to a process for the production of the bispecific binding molecule of the invention, a medical use of said bispecific binding molecule and a kit comprising said bispecific binding molecule.

[0002] . It is estimated that 1,529,560 men and women will be diagnosed with, and 569,490
15 men and women will die of, cancer in the US in 2010 (Howlader N, Noone AM, Krapcho M, Neyman N, Aminou R, Waldron W, Altekruse SF, Kosary CL, Ruhl J, Tatalovich Z, Cho H, Mariotto A, Eisner MP, Lewis DR, Chen HS, Feuer EJ, Cronin KA, Edwards BK (eds). SEER Cancer Statistics Review, 1975-2008, National Cancer Institute. Bethesda, MD, http://seer.cancer.gov/csr/1975_2008/, based on November 2010 SEER data submission,
20 posted to the SEER web site, 2011). As these numbers suggest, cancer is one of the major causes of morbidity and mortality in the developed world. For most epithelial cancers, which constitute the predominant majority of cancer cases, there is with few exceptions no curative treatment except for complete surgical resection in early stages. Disease progression in spite of repeated intensive standard treatments including surgery, radiotherapy and chemotherapy
25 leads to the substantial mortality stated above. Therefore, a high medical need exists for innovative treatment modalities to improve the outcome for these patient populations.

[0003] . The cell surface antigen defined by monoclonal antibody 5T4 is a 72-kD glycoprotein that is expressed by all types of trophoblasts as early as 9 weeks of development. In adult
30 tissues, the 5T4 expression is limited to a few specialized epithelial cell types but not detected in adult liver, lung, bronchus, heart, testis, ovary, brain or muscle. The antigen is selectively expressed by diverse tumour cell lines, including those of developmental origin. The molecular characteristics, the relatively restricted normal tissue distribution and expression by certain tumour cell types make this antigen worthy for use as a diagnostic
35 marker of malignancy (Hole & Stern, Br J Cancer. (1988) 57(3), 239-46).

[0004] . There is clear evidence for the involvement of 5T4 in cancer and cancer progression. High levels of 5T4 expression on the surface of cancer cells have been shown for ovarian cancer (Wrigley E, McGown AT, Rennison J, et al. 5T4 oncofetal antigen expression in ovarian carcinoma. *Int J Gynecol Cancer*. 1995;5:269-274), colon cancer
5 (Starzynska T, Marsh PJ, Schofield PF, Roberts SA, Myers KA, Stern PL. Prognostic significance of 5T4 oncofetal antigen expression in colorectal carcinoma. *Br J Cancer*. 1994;69:899-902), cancer of the cervix (Jones H, Roberts G, Hole N, McDicken IW, Stern P. Investigation of expression of 5T4 antigen in cervical cancer. *Br J Cancer*. 1990;6:69-100), gastric cancer (Starzynska T, Rahi V, Stern PL. The expression of 5T4 antigen in colorectal
10 and gastric carcinoma. *Br J Cancer*. 1992;66:867-869 and above) and lung cancer (Forsberg G, Ohlsson L, Brodin T, et al. Therapy of human non-small-cell lung carcinoma using antibody targeting of modified superantigen. *Br J Cancer*. 2001;85:129-136.). An association with disease progression, namely metastasis, was also demonstrated (Mulder WM, Stern PL, Stukart MJ, et al. Low intercellular adhesion molecule 1 and high 5T4 expression on tumor
15 cells correlate with reduced disease-free survival in colorectal carcinoma patients. *Clin Cancer*. 1997;3:1923-1930 and Starzynska T, Wiechowska-Kozłowska A, Marlicz K, et al. 5T4 oncofetal antigen in gastric carcinoma and its clinical significance. *Eur J Gastroenterol Hepatol*. 1998;10:479-484. and Naganuma H, Kono K, Mori Y, et al. Oncofetal antigen 5T4 expression as a prognostic factor in patients with gastric cancer. *Anticancer Res*.
20 2002;22:1033-1038). Since expression of 5T4 on normal tissues in contrast to cancerous lesions is restricted (Ali A, Langdon J, Stern PL, Partidge M. The pattern of expression of 5T4 oncofoetal antigen on normal, dysplastic and malignant oral mucosa. *Oral Oncol*. 2001;37:57-64), 5T4 has been suggested as a suitable target antigen for targeted cancer therapy (Woods AM, Wang WW, Shaw DM, et al. Characterization of the murine 5T4
25 oncofoetal antigen: a target for immunotherapy in cancer. *Biochem J*. 2002;366:353-365 and Hole N, Stern PL. Isolation and characterization of 5T4, A tumor-associated antigen. *Int J Cancer*. 1990;45:179-184).

[0005] . Antibody-based cancer therapies require a target antigen firmly bound to the surface
30 of cancer cells in order to be active. By binding to the surface target, the antibody can directly or indirectly deliver a deadly signal to the cancer cell. As required for an ideal treatment scenario, the target antigen 5T4 is abundantly present and accessible on specific cancer cells and is absent, shielded or much less abundant on normal cells.

35 **[0006]** . Yet, while the 5T4 antigen was identified as an oncology target more than 20 years ago, no anti-5T4 immunotherapy has been approved for treating cancer. Accordingly, there is a clear need for an effective therapeutic targeting this antigen.

[0007] . Accordingly, there is provided herewith means and methods for the solution of this problem in the form of a bispecific molecule binding with one domain to cytotoxic cells, i.e. cytotoxic T cells, and with a second binding domain to 5T4 on the surface of tumor cells.

5 **[0008]** . In one aspect, the present invention provides in a first embodiment a bispecific binding molecule comprising a first and a second binding domain,

(a) wherein the first binding domain is capable of binding to the epitope cluster 4 of 5T4; and

(b) wherein the second binding domain is capable of binding to the CD3 receptor complex
10 on T cells;

wherein the epitope cluster 4 corresponds to extracellular protein domain of 5T4 formed by amino acid residues 170 to 222 of the human sequence as depicted in SEQ ID NO: 2.

[0009] . The 5T4 onco-fetal antigen is a 72 kDa leucine-rich repeat (LRR) glycoprotein that
15 belongs to the group of type 1 transmembrane proteins. Moreover, the 5T4 antigen is a highly N-glycosylated protein with seven putative consensus N-linked glycosylation sites in the extracellular domain (Shaw et al. Biochem. J. 363 (2002) 137-145).

[0010] . The mature extracellular 5T4 antigen can be structured by its amino acid based
20 elements: An N-terminal serine-rich repeat (SRR; 22 aa), followed by a 36 aa long undefined area containing four cysteines, three adjacent leucine-rich repeats (LLR 1 – 3) of 24, 24, and 23 amino acids in length, respectively, a hydrophilic segment of 45 aa followed by three more leucine-rich repeats (LRR 4 – 5) of 24, 24, and 1+23 amino acids in length, respectively, a second undefined segment of 53 aa with four more cysteines which is finally followed by a
25 last leucine-rich repeat (LRR 7) of 22+4 amino acids in length (see Figure 1 for an illustration of the structure of 5T4). Since no crystal structure is available for the 5T4 antigen, a hypothetical structure is depicted in Figure 1, based on the structural comparison to the crystal structure of leucine-rich repeats from hagfish protein, in which each leucine-rich repeat forms one 23 aa long 360 degree bend of a helix (Kim et al. J. Biol. Chem. 282 (2007)
30 6726-6732); three LLRs in a row can thus form a small tube or barrel. A more complex structure would also be possible by interaction of the cysteines localized in the two structurally undefined segments, which might cause bending of the whole extracellular region possibly bringing the N-terminus close to the cell membrane. We have used N-terminal sequencing of human 5T4 to identify amino acid T35 in the immature protein to be the most
35 N-terminal amino acid of the mature protein after cleavage of the N-terminal leader peptide. A hypothetical structure of 5T4 which was used to design the experiments shown in the examples is depicted in Figure 1.

[0011] . The redirected lysis of target cells via the recruitment of T cells by bispecific molecules involves cytolytic synapse formation and delivery of perforin and granzymes. The engaged T cells are capable of serial target cell lysis, and are not affected by immune escape mechanisms interfering with peptide antigen processing and presentation, or clonal T cell differentiation. For the tumor antigen MCSP (Melanoma associated Chondroitin Sulfate Proteoglycan) it was described that despite similar binding affinity to MCSP, respective bispecific antibody molecules greatly differed in their potency of redirected lysis of CHO target cells stably transfected with full-length human MCSP. An epitope distance effect was corroborated indicating that the position of an epitope in a cell surface antigen is an important factor for bispecific antibody molecules potency, i.e. target cell lysis by membrane proximally-binding bispecific antibody molecules improved upon omission of more distal MCSP domains (Blümel et al. Cancer Immunol Immunother. 2010; 59(8), 1197). So informed, the person skilled in the art seeking to create a potent bispecific antibody would be biased to search for binders with high affinity for epitopes of 5T4 which are near the membrane.

[0012] . Moreover, the practitioner would expect that, for the generation of bispecific binding molecules for the elimination of target cells by effector cells (by the engagement of the effector and target cells), the potency of a compound should increase when the affinity of the compound to the target structure is increased.

[0013] . In contrast to the expectation of the field, it was surprisingly discovered that for binding molecules for 5T4 with comparable high affinity in the nM range for membrane proximal (epitope cluster 7) and membrane distal (epitope cluster 4 and 2/4) in a bispecific context, binders for the membrane distal epitope cluster show significantly higher potency in the lysis of 5T4 expressing target cells than those which bind to 5T4 at an epitope more proximal to the membrane. This is especially surprising since the affinity of the binder for 5T4 was not determined using artificial systems with recombinant antigen expression (e.g. immobilization of a chip) but by Scatchard using viable cells which naturally express the 5T4 antigen.

[0014] . It must be noted that as used herein, the singular forms “a”, “an”, and “the”, include plural references unless the context clearly indicates otherwise. Thus, for example, reference to “a reagent” includes one or more of such different reagents and reference to “the method” includes reference to equivalent steps and methods known to those of ordinary skill in the art that could be modified or substituted for the methods described herein.

Unless otherwise indicated, the term "at least" preceding a series of elements is to be understood to refer to every element in the series. Those skilled in the art will recognize, or

be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments of the invention described herein. Such equivalents are intended to be encompassed by the present invention.

[0015] . The term "and/or" wherever used herein includes the meaning of "and", "or" and "all or any other combination of the elements connected by said term".

[0016] . The term "about" or "approximately" as used herein means within 20%, preferably within 10%, and more preferably within 5% of a given value or range.

[0017] . Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integer or step. When used herein the term "comprising" can be substituted with the term "containing" or "including" or sometimes when used herein with the term "having".

[0018] . When used herein "consisting of" excludes any element, step, or ingredient not specified in the claim element. When used herein, "consisting essentially of" does not exclude materials or steps that do not materially affect the basic and novel characteristics of the claim.

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

[0019] . The term "binding domain" characterizes in connection with the present invention a domain of a polypeptide which specifically binds/interacts with a given target epitope (capable of binding to/interacting with a given target epitope). An "epitope" is antigenic and thus the term epitope is sometimes also referred to herein as "antigenic structure" or "antigenic determinant". Thus, the binding domain is an "antigen-interaction-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". In one example, said polypeptide which specifically binds/interacts with a given target epitope is an antibody and said domain is a VH and/or VL region of an antibody.

[0020] . The term "epitope" refers to a site on an antigen to which a binding domain, such as an antibody or immunoglobulin or derivative of an antibody or immunoglobulin, specifically binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. A "linear epitope" is an epitope where an amino acid primary sequence comprises the epitope recognized. A linear epitope typically

includes at least 3, and more usually, at least 5, for example, about 8 to about 10 amino acids in a unique sequence.

[0021] . A "conformational epitope", in contrast to a linear epitope, is an epitope wherein the primary sequence of the amino acids comprising the epitope is not the sole defining component of the epitope recognized (e.g., an epitope wherein the primary sequence of amino acids is not necessarily recognized by the antibody defining the epitope). Typically a conformational epitope comprises an increased number of amino acids relative to a linear epitope. With regard to recognition of conformational epitopes, the binding domain recognizes a 3-dimensional structure of the antigen, preferably a peptide or protein or fragment thereof (in the context of the present invention, the antigen is for one of the binding domains the 5T4 protein). For example, when a protein molecule folds to form a three dimensional structure, certain amino acids and/or the polypeptide backbone forming the conformational epitope become juxtaposed enabling the antibody to recognize the epitope. Methods of determining conformation of epitopes include but are not limited to, for example, x-ray crystallography 2-dimensional nuclear magnetic resonance spectroscopy and site-directed spin labeling and electron paramagnetic resonance spectroscopy. Moreover, the provided example describe a further method to test whether a given binding domain binds to one or more epitope cluster of a given protein

[0022] . As used herein, the term "epitope cluster" denotes the entirety of epitopes lying in a defined contiguous stretch of an antigen.

[0023] . In one aspect, a human 5T4 antigen binding ligand (e.g. a scFv) binds to one specific epitope cluster and significantly loses binding capability to the antigen when the respective epitope cluster in the human 5T4 protein is exchanged with the respective epitope cluster of a non-primate (e.g. murine) 5T4 antigen. A method to test this loss of binding due to exchange with the respective epitope cluster of a non-human (e.g. murine) 5T4 antigen is described in the appended examples 1 to 3.

[0024] . The terms "specifically recognizing", "directed to" and "reacting with" mean in accordance with this invention that a binding domain 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 epitope. Such binding may be exemplified by the specificity of a "lock-and-key-principle".

[0025] . As used herein, the terms "specifically interacting", "specifically binding" or "specifically binds" mean that a binding domain exhibits appreciable affinity for a particular protein or antigen and, generally, does not exhibit significant cross-reactivity with other proteins or antigens. "Appreciable" or preferred binding includes binding with an affinity of 10^{-6} M (KD) or higher. Preferably, binding is considered specific when binding affinity is about 10^{-11} to 10^{-8} M, preferably of about 10^{-11} to 10^{-9} M. If necessary, nonspecific binding can be reduced without substantially affecting specific binding by varying the binding conditions. Whether binding domains specifically react or bind with a target can be tested readily by, *inter alia*, comparing the reaction of one of said binding domains with a target protein or antigen with the reaction of said binding domains with other proteins or antigens.

[0026] . Specific binding is believed to be effected by specific motifs in the amino acid sequence of the binding domain and the antigen. Thus, binding is achieved as a result of their primary, secondary or tertiary structure as well as the result of secondary modifications of said structures. 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 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. In one example, a binding domain is an antibody.

[0027] . The term "does not essentially bind" means that a binding domain of the bispecific binding molecule of the present invention does not bind another protein, i.e., shows a cross-reactivity of less than 30%, preferably 20%, more preferably 10%, particularly preferably less than 9, 8, 7, 6 or 5% with another protein.

[0028] . The terms "cross-species specificity", "cross-species recognition" and "interspecies specificity" as used herein denote a binding domain's ability to bind to the same target molecule in humans and non-human species, preferably in primate species. Thus, "cross-species specificity" or "interspecies specificity" describes interspecies reactivity to the same molecule X (e.g., 5T4 or CD3 ϵ) expressed in different species, but not to a molecule other than X (e.g., 5T4 or CD3 ϵ).

[0029] . In one aspect, the cross-species specificity of the inventive binding domains is limited to human proteins and their corresponding analogs in primates. For example, it is apparent that the human 5T4 amino acid sequence (SEQ ID NO: 2) is quite similar to the

cynomolgus (SEQ ID NO: 4) or rhesus (SEQ ID NO: 6) 5T4 amino acid sequence. Thus, a binding domain against cynomolgus or rhesus 5T4 likely binds human 5T4.

[0030] . Accordingly, in one embodiment, a binding domain which binds to human 5T4, in particular to epitope cluster 4 of the extracellular protein domain of 5T4 formed by amino acid residues 170 to 222 of the human sequence as depicted in SEQ ID NO: 1, also binds to cynomolgus 5T4, in particular to epitope cluster 4 of the extracellular protein domain of 5T4 formed by amino acid residues 170 to 222 of the cynomolgus sequence as depicted in SEQ ID NO: 4.

[0031] . Proteins (including fragments thereof, preferably biologically active fragments, and peptides, usually having less than 30 amino acids) comprise one or more amino acids coupled to each other via a covalent peptide bond (resulting in a chain of amino acids). The term "polypeptide" as used herein describes a group of molecules, which consist of more than 30 amino acids. Polypeptides may further form multimers such as dimers, trimers and higher oligomers, i.e. consisting of more than one polypeptide molecule. Polypeptide molecules forming such dimers, trimers etc. may be identical or non-identical. The corresponding higher order structures of such multimers are, consequently, termed homo- or heterodimers, homo- or heterotrimers etc. An example for a heteromultimer is an antibody molecule, which, in its naturally occurring form, consists of two identical light polypeptide chains and two identical heavy polypeptide chains. The terms "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 the like. Such modifications are well known in the art.

[0032] . In one embodiment, a first binding domain of a bispecific binding molecule is capable of binding to epitope cluster 2 and epitope cluster 4 of 5T4, wherein the epitope cluster 2 corresponds to extracellular protein domain of 5T4 formed by amino acid residues 78 to 138 of the human sequence as depicted in SEQ ID NO: 2.

[0033] . As noted herein above, in one aspect, the first binding domain of the bispecific binding molecule is capable of binding to human and *Callithrix jacchus*, *Saguinus oedipus* or *Saimiri sciureus* 5T4 and/or the second binding domain is capable of binding to human and *Callithrix jacchus*, *Saguinus Oedipus* or *Saimiri sciureus* CD3 epsilon. According to this embodiment, one or both binding domains of the bispecific binding molecule of the invention are cross-species specific for members of the mammalian order of primates.

[0034] . In another embodiment, the affinity of the first binding domain for human 5T4 is ≤ 15 nM (preferably ≤ 10 nM).

Bispecific binding molecules of the invention binding to an epitope in epitope cluster 4 or epitope cluster 2 and 4 showing this high affinity for 5T4 have shown superior cytotoxic activity over bispecific binding molecules binding to an epitope in epitope cluster 7 with affinities in the same range.

[0035] . In one embodiment, the first or the second binding domain is derived from an antibody. In another, both binding domains are derived from an antibody.

[0036] . The definition of the term “antibody” includes embodiments such as monoclonal, chimeric, single chain, humanized and human 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 such as domain antibodies or nanobodies, single variable domain antibodies or immunoglobulin single variable domain comprising merely one variable domain, which might be VHH, VH or VL, that specifically bind an antigen or epitope independently of other V regions or domains; see, for example, Harlow and Lane (1988) and (1999), loc. cit.; Kontermann and Dübel, Antibody Engineering, Springer, 2nd ed. 2010 and Little, Recombinant Antibodies for Immunotherapy, Cambridge University Press 2009. 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.

[0037] . Various procedures are known in the art and may be used for the production of such antibodies and/or fragments. Thus, (antibody) derivatives can be produced by peptidomimetics. Further, techniques described for the production of single chain antibodies (see, inter alia, US Patent 4,946,778, Kontermann and Dübel (2010), loc. cit. and Little(2009), loc. cit.) can be adapted to produce single chain antibodies specific for elected polypeptide(s). Also, transgenic animals may be used to express humanized 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 (Schier, Human Antibodies Hybridomas 7 (1996), 97-105; Malmborg, 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
5 constructs which may be transfected and/or transduced via, inter alia, viruses or plasmid vectors.

[0038] . Furthermore, the term "antibody" as employed in the invention also relates to derivatives or variants of the antibodies described herein which display the same specificity
10 as the described antibodies. Examples of "antibody variants" include humanized variants of non- human antibodies, "affinity matured" antibodies (see, e.g. Hawkins et al. J. Mol. Biol. 254, 889-896 (1992) and Lowman et al., Biochemistry 30, 10832- 10837 (1991)) and antibody mutants with altered effector function (s) (see, e.g., US Patent 5, 648, 260, Kontermann and Dübel (2010), loc. cit. and Little(2009), loc. cit.).

15 The terms "antigen-binding domain", "antigen-binding fragment" and "antibody binding region" when used herein refer to a part of an antibody molecule that comprises amino acids responsible for the specific binding between antibody and antigen. The part of the antigen that is specifically recognized and bound by the antibody is referred to as the "epitope" as described herein above. As mentioned above, an antigen-binding domain may typically
20 comprise an antibody light chain variable region (VL) and an antibody heavy chain variable region (VH); however, it does not have to comprise both. Fd fragments, for example, have two VH regions and often retain some antigen-binding function of the intact antigen-binding domain. Examples of antigen-binding fragments of an antibody include (1) a Fab fragment, a monovalent fragment having the VL, VH, CL and CH1 domains; (2) a F(ab')₂ fragment, a
25 bivalent fragment having two Fab fragments linked by a disulfide bridge at the hinge region; (3) a Fd fragment having the two VH and CH1 domains; (4) a Fv fragment having the VL and VH domains of a single arm of an antibody, (5) a dAb fragment (Ward et al., (1989) Nature 341 :544-546), which has a VH domain; (6) an isolated complementarity determining region (CDR), and (7) a single chain Fv (scFv). Although the two domains of the Fv fragment, VL
30 and VH are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Huston et al. (1988) Proc. Natl. Acad. Sci USA 85:5879-5883). These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the
35 fragments are evaluated for function in the same manner as are intact antibodies.

[0039] . The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, i.e., the individual antibodies comprising the population are identical except for possible naturally occurring mutations and/or post- translation modifications (e.g., isomerizations, amidations) that may be present in minor amounts. Monoclonal antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. In addition to their specificity, the monoclonal antibodies are advantageous in that they are synthesized by the hybridoma culture, uncontaminated by other immunoglobulins. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. For example, the monoclonal antibodies to be used in accordance with the present invention may be made by the hybridoma method first described by Kohler et al., Nature, 256: 495 (1975), or may be made by recombinant DNA methods (see, e.g., U. S. Patent No. 4,816, 567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson et al., Nature, 352: 624-628 (1991) and Marks et al., J. Mol. Biol., 222: 581-597 (1991), for example.

The monoclonal antibodies herein specifically include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain (s) is (are) identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies, so long as they exhibit the desired biological activity (U. S. Patent No. 4,816, 567; Morrison et al., Proc. Natl. Acad. Sci. USA, 81: 6851-6855 (1984)). Chimeric antibodies of interest herein include "primitized" antibodies comprising variable domain antigen-binding sequences derived from a non-human primate (e.g., Old World Monkey, Ape etc.) and human constant region sequences.

[0040] . "Humanized" forms of non-human (e.g., murine) antibodies are chimeric immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F (ab')₂ or other antigen-binding subsequences of antibodies) of mostly human sequences, which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from a hypervariable region (also CDR) of the recipient are replaced by residues from a hypervariable region of a non-human species (donor antibody) such as mouse, rat or rabbit

having the desired specificity, affinity, and capacity. In some instances, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, "humanized antibodies" as used herein may also comprise residues which are found neither in the recipient antibody nor the donor antibody. These modifications
5 are made to further refine and optimize antibody performance. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones et al., Nature, 321: 522-525 (1986); Reichmann et al., Nature, 332: 323-329 (1988); and Presta, Curr. Op. Struct. Biol., 2: 593-596 (1992).

10 **[0041]** . The term "human antibody" includes antibodies having variable and constant regions corresponding substantially to human germline immunoglobulin sequences known in the art, including, for example, those described by Kabat et al. (See Kabat, et al. (1991) loc. cit.). The human antibodies of the invention may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific
15 mutagenesis in vitro or by somatic mutation in vivo), for example in the CDRs, and in particular, CDR3. The human antibody can have at least one, two, three, four, five, or more positions replaced with an amino acid residue that is not encoded by the human germline immunoglobulin sequence.

[0042] . As used herein, "in vitro generated antibody" refers to an antibody where all or part
20 of the variable region (e.g., at least one CDR) is generated in a non-immune cell selection (e.g., an in vitro phage display, protein chip or any other method in which candidate sequences can be tested for their ability to bind to an antigen). This term thus preferably excludes sequences generated by genomic rearrangement in an immune cell.

25 **[0043]** . A "bispecific" or "bifunctional antibody" is an artificial hybrid antibody having two different heavy/light chain pairs and two different binding sites. Bispecific antibodies can be produced by a variety of methods including fusion of hybridomas or linking of Fab' fragments. See, e.g., Songsivilai & Lachmann, Clin. Exp. Immunol. 79:315-321 (1990). Numerous methods known to those skilled in the art are available for obtaining antibodies or antigen-
30 binding fragments thereof. For example, antibodies can be produced using recombinant DNA methods (U.S. Patent 4,816,567). Monoclonal antibodies may also be produced by generation of hybridomas (see e.g., Kohler and Milstein (1975) Nature, 256: 495-499) in accordance with known methods. Hybridomas formed in this manner are then screened using standard methods, such as enzyme-linked immunosorbent assay (ELISA) and surface
35 plasmon resonance (BIAcore™) analysis, to identify one or more hybridomas that produce an antibody that specifically binds with a specified antigen. Any form of the specified antigen

may be used as the immunogen, e.g., recombinant antigen, naturally occurring forms, any variants or fragments thereof, as well as antigenic peptide thereof.

One exemplary method of making antibodies includes screening protein expression libraries, e.g., phage or ribosome display libraries. Phage display is described, for example, in Ladner
5 et al., U.S. Patent No. 5,223,409; Smith (1985) Science 228:1315-1317; Clackson et al. (1991) Nature, 352: 624-628.

[0044] . In addition to the use of display libraries, the specified antigen can be used to immunize a non-human animal, e.g., a rodent, e.g., a mouse, hamster, or rat. In one embodiment, the non-human animal includes at least a part of a human immunoglobulin
10 gene. For example, it is possible to engineer mouse strains deficient in mouse antibody production with large fragments of the human Ig loci. Using the hybridoma technology, antigen-specific monoclonal antibodies derived from the genes with the desired specificity may be produced and selected. See, e.g., XENOMOUSE™, Green et al. (1994) Nature Genetics 7:13-21, US 2003- 0070185, WO 96/34096, and WO96/33735.

15 **[0045]** . A monoclonal antibody can be obtained from a non-human animal, and then modified, e.g., humanized, deimmunized, chimeric, may be produced using recombinant DNA techniques known in the art. A variety of approaches for making chimeric antibodies have been described. See e.g., Morrison et al., Proc. Natl. Acad. Sci. U.S.A. 81:6851 , 1985; Takeda et al., Nature 314:452, 1985, Cabilly et al., U.S. Patent No. 4,816,567; Boss et al.,
20 U.S. Patent No. 4,816,397; Tanaguchi et al., EP 171496; EP 173494, GB 2177096. Humanized antibodies may also be produced, for example, using transgenic mice that express human heavy and light chain genes, but are incapable of expressing the endogenous mouse immunoglobulin heavy and light chain genes. Winter describes an
25 exemplary CDR-grafting method that may be used to prepare the humanized antibodies described herein (U.S. Patent No. 5,225,539). All of the CDRs of a particular human antibody may be replaced with at least a portion of a non-human CDR, or only some of the CDRs may be replaced with non-human CDRs. It is only necessary to replace the number of CDRs required for binding of the humanized antibody to a predetermined antigen.

30 Humanized antibodies or fragments thereof can be generated by replacing sequences of the Fv variable domain that are not directly involved in antigen binding with equivalent sequences from human Fv variable domains. Exemplary methods for generating humanized antibodies or fragments thereof are provided by Morrison (1985) Science 229:1202-1207; by Oi et al. (1986) BioTechniques 4:214; and by US 5,585,089; US 5,693,761; US 5,693,762;
35 US 5,859,205; and US 6,407,213. Those methods include isolating, manipulating, and expressing the nucleic acid sequences that encode all or part of immunoglobulin Fv variable domains from at least one of a heavy or light chain. Such nucleic acids may be obtained from

a hybridoma producing an antibody against a predetermined target, as described above, as well as from other sources. The recombinant DNA encoding the humanized antibody molecule can then be cloned into an appropriate expression vector.

[0046] . A humanized antibody can be optimized by the introduction of conservative substitutions, consensus sequence substitutions, germline substitutions and/or back mutations. Such altered immunoglobulin molecules can be made by any of several techniques known in the art, (e.g., Teng et al., Proc. Natl. Acad. Sci. U.S.A., 80: 7308-7312, 1983; Kozbor et al, Immunology Today, 4: 7279, 1983; Olsson et al., Meth. Enzymol., 92: 3-16, 1982, and may be made according to the teachings of EP 239 400).

[0047] . An antibody or fragment thereof may also be modified by specific deletion of human T cell epitopes or "deimmunization" by the methods disclosed in WO 98/52976 and WO 00/34317. Briefly, the heavy and light chain variable domains of an antibody can be analyzed for peptides that bind to MHC Class II; these peptides represent potential T-cell epitopes (as defined in WO 98/52976 and WO 00/34317). For detection of potential T-cell epitopes, a computer modeling approach termed "peptide threading" can be applied, and in addition a database of human MHC class II binding peptides can be searched for motifs present in the VH and VL sequences, as described in WO 98/52976 and WO 00/34317. These motifs bind to any of the 18 major MHC class II DR allotypes, and thus constitute potential T cell epitopes. Potential T-cell epitopes detected can be eliminated by substituting small numbers of amino acid residues in the variable domains, or preferably, by single amino acid substitutions. Typically, conservative substitutions are made. Often, but not exclusively, an amino acid common to a position in human germline antibody sequences may be used. Human germline sequences, e.g., are disclosed in Tomlinson, et al. (1992) J. Mol. Biol. 227:776-798; J. Mol. Biol. 227:776-798; Cook, G. P. et al. (1995) Immunol. Today Vol. 16 (5): 237-242; and Tomlinson et al. (1995) EMBO J. 14: 14:4628-4638. The V BASE directory provides a comprehensive directory of human immunoglobulin variable region sequences (compiled by Tomlinson, LA. et al. MRC Centre for Protein Engineering, Cambridge, UK). These sequences can be used as a source of human sequence, e.g., for framework regions and CDRs. Consensus human framework regions can also be used, e.g., as described in U.S. Patent No. 6,300,064.

[0048] . The pairing of a VH and VL together forms a single antigen-binding site. The CH domain most proximal to VH is designated as CH1. Each L chain is linked to an H chain by one covalent disulfide bond, while the two H chains are linked to each other by one or more disulfide bonds depending on the H chain isotype. The VH and VL domains consist of four regions of relatively conserved sequences called framework regions (FR1, FR2, FR3, and FR4), which form a scaffold for three regions of hypervariable sequences (complementarity

determining regions, CDRs). The CDRs contain most of the residues responsible for specific interactions of the antibody with the antigen. CDRs are referred to as CDR 1, CDR2, and CDR3. Accordingly, CDR constituents on the heavy chain are referred to as H1, H2, and H3, while CDR constituents on the light chain are referred to as L1, L2, and L3.

5 **[0049]** . The term "variable" refers to the portions of the immunoglobulin domains that exhibit variability in their sequence and that are involved in determining the specificity and binding affinity of a particular antibody (i.e., the "variable domain(s)"). Variability is not evenly distributed throughout the variable domains of antibodies; it is concentrated in sub-domains of each of the heavy and light chain variable regions. These sub-domains are called
10 "hypervariable" regions or "complementarity determining regions" (CDRs). The more conserved (i.e., non-hypervariable) portions of the variable domains are called the "framework" regions (FRM). The variable domains of naturally occurring heavy and light chains each comprise four FRM regions, largely adopting a β - sheet configuration, connected by three hypervariable regions, which form loops connecting, and in some cases forming part
15 of, the β -sheet structure. The hypervariable regions in each chain are held together in close proximity by the FRM and, with the hypervariable regions from the other chain, contribute to the formation of the antigen- binding site (see Kabat et al., loc. cit.). The constant domains are not directly involved in antigen binding, but exhibit various effector functions, such as, for example, antibody- dependent, cell-mediated cytotoxicity and complement activation.

20 **[0050]** . It is also preferred for the bispecific binding molecule of the invention that first and the second domain form a molecule that is selected from the group of (scFv)₂, (single domain mAb)₂, scFv-single domain mAb, diabody or oligomeres thereof.

25 **[0051]** . The terms "CDR", and its plural "CDRs", refer to a complementarity determining region (CDR) of which three make up the binding character of a light chain variable region (CDRL1, CDRL2 and CDRL3) and three make up the binding character of a heavy chain variable region (CDRH1, CDRH2 and CDRH3). CDRs contribute to the functional activity of an antibody molecule and are separated by amino acid sequences that comprise scaffolding
30 or framework regions. The exact definitional CDR boundaries and lengths are subject to different classification and numbering systems. CDRs may therefore be referred to by Kabat, Chothia, contact or any other boundary definitions, including the numbering system described herein. Despite differing boundaries, each of these systems has some degree of overlap in what constitutes the so called "hypervariable regions" within the variable
35 sequences. CDR definitions according to these systems may therefore differ in length and boundary areas with respect to the adjacent framework region. See for example Kabat, Chothia, and/or MacCallum et al., (Kabat et al., loc. cit.; Chothia et al., J. Mol. Biol, 1987,

196: 901; and MacCallum et al, J. Mol. Biol, 1996, 262: 732). However, the numbering in accordance with the so-called Kabat system is preferred.

[0052] . The term "amino acid" or "amino acid residue" typically refers to an amino acid having its art recognized definition such as an amino acid selected from the group consisting of: alanine (Ala or A); arginine (Arg or R); asparagine (Asn or N); aspartic acid (Asp or D);
5 cysteine (Cys or C); glutamine (Gln or Q); glutamic acid (Glu or E); glycine (Gly or G); histidine (His or H); isoleucine (Ile or I); leucine (Leu or L); lysine (Lys or K); methionine (Met or M); phenylalanine (Phe or F); proline (Pro or P); serine (Ser or S); threonine (Thr or T); tryptophan (Trp or W); tyrosine (Tyr or Y); and valine (Val or V), although modified, synthetic,
10 or rare amino acids may be used as desired. Generally, amino acids can be grouped as having a nonpolar side chain (e.g., Ala, Cys, Ile, Leu, Met, Phe, Pro, Val); a negatively charged side chain (e.g., Asp, Glu); a positively charged sidechain (e.g., Arg, His, Lys); or an uncharged polar side chain (e.g., Asn, Cys, Gln, Gly, His, Met, Phe, Ser, Thr, Trp, and Tyr).

[0053] . The term "hypervariable region" (also known as "complementarity determining regions" or CDRs) when used herein refers to the amino acid residues of an antibody which are (usually three or four short regions of extreme sequence variability) within the V-region domain of an immunoglobulin which form the antigen-binding site and are the main determinants of antigen specificity. There are at least two methods for identifying the CDR
15 residues: (1) An approach based on cross-species sequence variability (i. e., Kabat et al., loc. cit.); and (2) An approach based on crystallographic studies of antigen-antibody complexes (Chothia, C. et al., J. Mol. Biol. 196: 901-917 (1987)). However, to the extent that two residue identification techniques define regions of overlapping, but not identical regions, they can be combined to define a hybrid CDR. However, in general, the CDR residues are
20 preferably identified in accordance with the so-called Kabat (numbering) system.

[0054] . The term "framework region" refers to the art-recognized portions of an antibody variable region that exist between the more divergent (i.e., hypervariable) CDRs. Such framework regions are typically referred to as frameworks 1 through 4 (FR1, FR2, FR3, and
30 FR4) and provide a scaffold for the presentation of the six CDRs (three from the heavy chain and three from the light chain) in three dimensional space, to form an antigen-binding surface.

[0055] . Typically, CDRs form a loop structure that can be classified as a canonical structure.
35 The term "canonical structure" refers to the main chain conformation that is adopted by the antigen binding (CDR) loops. From comparative structural studies, it has been found that five of the six antigen binding loops have only a limited repertoire of available conformations.

Each canonical structure can be characterized by the torsion angles of the polypeptide backbone. Correspondent loops between antibodies may, therefore, have very similar three dimensional structures, despite high amino acid sequence variability in most parts of the loops (Chothia and Lesk, J. Mol. Biol., 1987, 196: 901; Chothia et al, Nature, 1989, 342: 877; 5 Martin and Thornton, J. Mol. Biol, 1996, 263: 800, each of which is incorporated by reference in its entirety). Furthermore, there is a relationship between the adopted loop structure and the amino acid sequences surrounding it. The conformation of a particular canonical class is determined by the length of the loop and the amino acid residues residing at key positions within the loop, as well as within the conserved framework (i.e., outside of the loop). 10 Assignment to a particular canonical class can therefore be made based on the presence of these key amino acid residues. The term "canonical structure" may also include considerations as to the linear sequence of the antibody, for example, as catalogued by Kabat (Kabat et al, loc. cit.). The Kabat numbering scheme (system) is a widely adopted standard for numbering the amino acid residues of an antibody variable domain in a 15 consistent manner and is the preferred scheme applied in the present invention as also mentioned elsewhere herein. Additional structural considerations can also be used to determine the canonical structure of an antibody. For example, those differences not fully reflected by Kabat numbering can be described by the numbering system of Chothia et al and/or revealed by other techniques, for example, crystallography and two or three- 20 dimensional computational modeling. Accordingly, a given antibody sequence may be placed into a canonical class which allows for, among other things, identifying appropriate chassis sequences {e.g., based on a desire to include a variety of canonical structures in a library). Kabat numbering of antibody amino acid sequences and structural considerations as described by Chothia et al., loc. cit. and their implications for construing canonical aspects of 25 antibody structure, are described in the literature.

[0056] . CDR3 is typically the greatest source of molecular diversity within the antibody-binding site. H3, for example, can be as short as two amino acid residues or greater than 26 amino acids. The subunit structures and three-dimensional configurations of different classes 30 of immunoglobulins are well known in the art. For a review of the antibody structure, see Antibodies: A Laboratory Manual, Cold Spring Harbor Laboratory, eds. Harlow et al., 1988. One of skill in the art will recognize that each subunit structure, e.g., a CH, VH, CL, VL, CDR, FR structure, comprises active fragments, e.g., the portion of the VH, VL, or CDR subunit the binds to the antigen, i.e., the antigen-binding fragment, or, e.g., the portion of the CH subunit 35 that binds to and/or activates, e.g., an Fc receptor and/or complement. The CDRs typically refer to the Kabat CDRs, as described in Sequences of Proteins of immunological Interest, US Department of Health and Human Services (1991), eds. Kabat et al. Another standard for

characterizing the antigen binding site is to refer to the hypervariable loops as described by Chothia. See, e.g., Chothia, et al. (1987; J. Mol. Biol. 227:799-817; and Tomlinson et al. (1995) EMBO J. 14: 4628-4638. Still another standard is the AbM definition used by Oxford Molecular's AbM antibody modeling software. See, generally, e.g., Protein Sequence and
5 Structure Analysis of Antibody Variable Domains. In: Antibody Engineering Lab Manual (Ed.: Duebel, S. and Kontermann, R., Springer-Verlag, Heidelberg). Embodiments described with respect to Kabat CDRs can alternatively be implemented using similar described relationships with respect to Chothia hypervariable loops or to the AbM-defined loops.

[0057] . The sequence of antibody genes after assembly and somatic mutation is highly
10 varied, and these varied genes are estimated to encode 10^{10} different antibody molecules (Immunoglobulin Genes, 2nd ed., eds. Jonio et al., Academic Press, San Diego, CA, 1995). Accordingly, the immune system provides a repertoire of immunoglobulins. The term "repertoire" refers to at least one nucleotide sequence derived wholly or partially from at least one sequence encoding at least one immunoglobulin. The sequence(s) may be generated by
15 rearrangement in vivo of the V, D, and J segments of heavy chains, and the V and J segments of light chains. Alternatively, the sequence(s) can be generated from a cell in response to which rearrangement occurs, e.g., in vitro stimulation. Alternatively, part or all of the sequence(s) may be obtained by DNA splicing, nucleotide synthesis, mutagenesis, and other methods, see, e.g., U.S. Patent 5,565,332. A repertoire may include only one
20 sequence or may include a plurality of sequences, including ones in a genetically diverse collection.

[0058] . In one embodiment, the first binding domain of a bispecific binding molecule comprises a VL region and a VH region, wherein the VH region comprises a CDR-H3
25 selected from the group consisting of SEQ ID NOs: **15, 25, 35, 49, 89, 99, 109, 119, 129, 143, 157 and 167.**

In another, the bispecific binding molecule of the invention comprises a VL region comprising CDR-L1, CDR-L2 and CDR-L3 and a VH region comprising CDR-H1, CDR-H2 and CDR-H3 selected from:

- 30 (a) CDR-L1 as depicted in SEQ ID NO. 16, CDR-L2 as depicted in SEQ ID NO. 17, CDR-L3 as depicted in SEQ ID NO. 18, CDR-H1 as depicted in SEQ ID NO. 13, CDR-H2 as depicted in SEQ ID NO. 14 and CDR-H3 as depicted in SEQ ID NO. 15;
- (b) CDR-L1 as depicted in SEQ ID NO. 26, CDR-L2 as depicted in SEQ ID NO. 17, CDR-L3 as depicted in SEQ ID NO. 28, CDR-H1 as depicted in SEQ ID NO. 23, CDR-H2 as
35 depicted in SEQ ID NO. 24 and CDR-H3 as depicted in SEQ ID NO. 25;

- (c) CDR-L1 as depicted in SEQ ID NO. 36, CDR-L2 as depicted in SEQ ID NO. 37, CDR-L3 as depicted in SEQ ID NO. 38, CDR-H1 as depicted in SEQ ID NO. 33, CDR-H2 as depicted in SEQ ID NO. 34 and CDR-H3 as depicted in SEQ ID NO. 35;
- (d) CDR-L1 as depicted in SEQ ID NO. 50, CDR-L2 as depicted in SEQ ID NO. 51, CDR-L3 as depicted in SEQ ID NO. 52, CDR-H1 as depicted in SEQ ID NO. 47, CDR-H2 as depicted in SEQ ID NO. 48 and CDR-H3 as depicted in SEQ ID NO. 49;
- (e) CDR-L1 as depicted in SEQ ID NO. 90, CDR-L2 as depicted in SEQ ID NO. 91, CDR-L3 as depicted in SEQ ID NO. 92, CDR-H1 as depicted in SEQ ID NO. 87, CDR-H2 as depicted in SEQ ID NO. 88 and CDR-H3 as depicted in SEQ ID NO. 89;
- (f) CDR-L1 as depicted in SEQ ID NO. 100, CDR-L2 as depicted in SEQ ID NO. 101, CDR-L3 as depicted in SEQ ID NO. 102, CDR-H1 as depicted in SEQ ID NO. 97, CDR-H2 as depicted in SEQ ID NO. 98 and CDR-H3 as depicted in SEQ ID NO. 99;
- (g) CDR-L1 as depicted in SEQ ID NO. 110, CDR-L2 as depicted in SEQ ID NO. 111, CDR-L3 as depicted in SEQ ID NO. 112, CDR-H1 as depicted in SEQ ID NO. 107, CDR-H2 as depicted in SEQ ID NO. 108 and CDR-H3 as depicted in SEQ ID NO. 109;
- (h) CDR-L1 as depicted in SEQ ID NO. 120, CDR-L2 as depicted in SEQ ID NO. 121, CDR-L3 as depicted in SEQ ID NO. 122, CDR-H1 as depicted in SEQ ID NO. 117, CDR-H2 as depicted in SEQ ID NO. 118 and CDR-H3 as depicted in SEQ ID NO. 119;
- (i) CDR-L1 as depicted in SEQ ID NO. 130, CDR-L2 as depicted in SEQ ID NO. 131, CDR-L3 as depicted in SEQ ID NO. 132, CDR-H1 as depicted in SEQ ID NO. 127, CDR-H2 as depicted in SEQ ID NO. 128 and CDR-H3 as depicted in SEQ ID NO. 129;
- (j) CDR-L1 as depicted in SEQ ID NO. 144, CDR-L2 as depicted in SEQ ID NO. 145, CDR-L3 as depicted in SEQ ID NO. 146, CDR-H1 as depicted in SEQ ID NO. 141, CDR-H2 as depicted in SEQ ID NO. 142 and CDR-H3 as depicted in SEQ ID NO. 143;
- (k) CDR-L1 as depicted in SEQ ID NO. 158, CDR-L2 as depicted in SEQ ID NO. 159, CDR-L3 as depicted in SEQ ID NO. 160, CDR-H1 as depicted in SEQ ID NO. 155, CDR-H2 as depicted in SEQ ID NO. 156 and CDR-H3 as depicted in SEQ ID NO. 157; and
- (l) CDR-L1 as depicted in SEQ ID NO. 168, CDR-L2 as depicted in SEQ ID NO. 169, CDR-L3 as depicted in SEQ ID NO. 170, CDR-H1 as depicted in SEQ ID NO. 165, CDR-H2 as depicted in SEQ ID NO. 166 and CDR-H3 as depicted in SEQ ID NO. 167.

[0059] . In another embodiment, the first binding domain of the bispecific binding molecule comprises a VL region selected from the group consisting of a VL region as depicted in SEQ ID NO: **12**, SEQ ID NO: **22**, SEQ ID NO: **32**, SEQ ID NO: **46**, SEQ ID NO: **86**, SEQ ID NO: **96**, SEQ ID NO: **106**, SEQ ID NO: **116**, SEQ ID NO: **126**, SEQ ID NO: **140**, SEQ ID NO: **154**, and SEQ ID NO: **164**.

[0060] . Alternatively, the first binding domain comprises a VH region selected from the group consisting of a VH region as depicted in SEQ ID NO: **11**, SEQ ID NO: **21**, SEQ ID NO: **31**, SEQ ID NO: **45**, SEQ ID NO: **85**, SEQ ID NO: **95**, SEQ ID NO: **105**, SEQ ID NO: **115**, SEQ ID NO: **125**, SEQ ID NO: **139**, SEQ ID NO: **153**, and SEQ ID NO: **163**.

[0061] . In one embodiment, the first binding domain of the bispecific binding molecule comprises a VL region and a VH region selected from the group consisting of:

- (a) a VL region as depicted in SEQ ID NO: 12 and a VH region as depicted in SEQ ID NO: 11;
- (b) a VL region as depicted in SEQ ID NO: 22 and a VH region as depicted in SEQ ID NO: 21;
- (c) a VL region as depicted in SEQ ID NO: 32 and a VH region as depicted in SEQ ID NO: 31;
- (d) a VL region as depicted in SEQ ID NO: 46 and a VH region as depicted in SEQ ID NO: 45;
- (e) a VL region as depicted in SEQ ID NO: 86 and a VH region as depicted in SEQ ID NO: 85;
- (f) a VL region as depicted in SEQ ID NO: 96 and a VH region as depicted in SEQ ID NO: 95;
- (g) a VL region as depicted in SEQ ID NO: 106 and a VH region as depicted in SEQ ID NO: 105;
- (h) a VL region as depicted in SEQ ID NO: 116 and a VH region as depicted in SEQ ID NO: 115;
- (i) a VL region as depicted in SEQ ID NO: 126 and a VH region as depicted in SEQ ID NO: 125;
- (j) a VL region as depicted in SEQ ID NO: 140 and a VH region as depicted in SEQ ID NO: 139;
- (k) a VL region as depicted in SEQ ID NO: 154 and a VH region as depicted in SEQ ID NO: 153; and
- (l) a VL region as depicted in SEQ ID NO: 164 and a VH region as depicted in SEQ ID NO: 163.

[0062] . In one example, the first binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: **10**, SEQ ID NO: **20**, SEQ ID NO: **30**, SEQ ID NO: **39**, SEQ ID NO: **44**, SEQ ID NO: **84**, SEQ ID NO: **94**, SEQ ID NO: **104**, SEQ ID NO:

114, SEQ ID NO: 124, SEQ ID NO: 133, SEQ ID NO: 138, SEQ ID NO: 147, SEQ ID NO: 152, and SEQ ID NO: 162.

[0063] . The bispecific binding molecule of the present invention is preferably an "isolated" bispecific binding molecule. "Isolated" when used to describe the bispecific binding molecule disclosed herein, means a bispecific binding molecule that has been identified, separated and/or recovered from a component of its production environment. Preferably, the isolated bispecific binding molecule is free of association with all other components from its production environment. Contaminant components of its production environment, such as that resulting from recombinant transfected cells, are materials that would typically interfere with diagnostic or therapeutic uses for the polypeptide, and may include enzymes, hormones, and other proteinaceous or non-proteinaceous solutes. In preferred embodiments, the bispecific binding molecule will be purified (1) to a degree sufficient to obtain at least 15 residues of N-terminal or internal amino acid sequence by use of a spinning cup sequenator, or (2) to homogeneity by SDS-PAGE under non-reducing or reducing conditions using Coomassie blue or, preferably, silver stain. Ordinarily, however, an isolated antibody will be prepared by at least one purification step.

[0064] . Amino acid sequence modifications of the bispecific binding molecules described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. Amino acid sequence variants of the bispecific binding molecules are prepared by introducing appropriate nucleotide changes into the bispecific binding molecules nucleic acid, or by peptide synthesis.

[0065] . Such modifications include, for example, deletions from, and/or insertions into, and/or substitutions of, residues within the amino acid sequences of the bispecific binding molecules. Any combination of deletion, insertion, and substitution is made to arrive at the final construct, provided that the final construct possesses the desired characteristics. The amino acid changes also may alter post-translational processes of the bispecific binding molecules, such as changing the number or position of glycosylation sites. Preferably, 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acids may be substituted in a CDR, while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be substituted in the FRs. The substitutions are preferably conservative substitutions as described herein. Additionally or alternatively, 1, 2, 3, 4, 5, or 6 amino acids may be inserted or deleted in each of the CDRs (of course, dependent on their length), while 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, or 25 amino acids may be inserted or deleted in each of the FRs.

[0066] . A useful method for identification of certain residues or regions of the bispecific binding molecules that are preferred locations for mutagenesis is called "alanine scanning mutagenesis" as described by Cunningham and Wells in Science, 244: 1081-1085 (1989).

Here, a residue or group of target residues within the bispecific binding molecule is/are identified (e.g. charged residues such as arg, asp, his, lys, and glu) and replaced by a neutral or negatively charged amino acid (most preferably alanine or polyalanine) to affect the interaction of the amino acids with epitope.

5 **[0067]** . Those amino acid locations demonstrating functional sensitivity to the substitutions then are refined by introducing further or other variants at, or for, the sites of substitution. Thus, while the site for introducing an amino acid sequence variation is predetermined, the nature of the mutation per se need not be predetermined. For example, to analyze the performance of a mutation at a given site, ala scanning or random mutagenesis is conducted
10 at a target codon or region and the expressed bispecific binding molecule variants are screened for the desired activity.

[0068] . Preferably, amino acid sequence insertions include amino-and/or carboxyl-terminal fusions ranging in length from one, two, three, four, five, six, seven, eight, nine or ten residues to polypeptides containing a hundred or more residues, as well as intrasequence
15 insertions of single or multiple amino acid residues. An insertional variant of the bispecific binding molecule include the fusion to the N-or C-terminus of the antibody to an enzyme or a fusion to a polypeptide which increases the serum half-life of the antibody.

[0069] . Another type of variant is an amino acid substitution variant. These variants have preferably at least one, two, three, four, five, six, seven, eight, nine or ten amino acid
20 residues in the bispecific binding molecule replaced by a different residue. The sites of greatest interest for substitutional mutagenesis include the CDRs of the heavy and/or light chain, in particular the hypervariable regions, but FR alterations in the heavy and/or light chain are also contemplated.

25 **[0070]** . For example, if a CDR sequence encompasses 6 amino acids, it is envisaged that one, two or three of these amino acids are substituted. Similarly, if a CDR sequence encompasses 15 amino acids it is envisaged that one, two, three, four, five or six of these amino acids are substituted.

[0071] . Generally, if amino acids are substituted in one or more or all of the CDRs of the
30 heavy and/or light chain, it is preferred that the then-obtained "substituted" sequence is at least 60%, more preferably 65%, even more preferably 70%, particularly preferable 75%, more particularly preferable 80% identical to the "original" CDR sequence. This means that it is dependent of the length of the CDR to which degree it is identical to the "substituted" sequence. For example, a CDR having 5 amino acids is preferably 80% identical to its
35 substituted sequence in order to have at least one amino acid substituted. Accordingly, the CDRs of the bispecific binding molecule may have different degrees of identity to their substituted sequences, e.g., CDRL1 may have 80%, while CDRL3 may have 90%.

[0072] . Preferred substitutions (or replacements) are conservative substitutions. However, any substitution (including non-conservative substitution or one or more from the “exemplary substitutions listed in Table 1, below) is envisaged as long as the bispecific binding molecule retains its capability to bind to 5T4 via the first binding domain and to CD3 ϵ via the second binding domain and/or its CDRs have an identity to the then substituted sequence (at least 60%, more preferably 65%, even more preferably 70%, particularly preferable 75%, more particularly preferable 80% identical to the “original” CDR sequence).

[0073] . Conservative substitutions are shown in Table I under the heading of "preferred substitutions". If such substitutions result in a change in biological activity, then more substantial changes, denominated "exemplary substitutions" in Table 1, or as further described below in reference to amino acid classes, may be introduced and the products screened for a desired characteristic.

TABLE I Amino Acid Substitutions

Original	Exemplary Substitutions	Preferred Substitutions
Ala (A)	val; leu; ile	Val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp; lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe;	leu
Leu (L)	norleucine; ile; val; met; ala;	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	Phe
Val (V)	ile; leu; met; phe; ala;	leu

[0074] . Substantial modifications in the biological properties of the binding molecule of the present invention are accomplished by selecting substitutions that differ significantly in their effect on maintaining (a) the structure of the polypeptide backbone in the area of the substitution, for example, as a sheet or helical conformation, (b) the charge or hydrophobicity of the molecule at the target site, or (c) the bulk of the side chain. Naturally occurring residues are divided into groups based on common side-chain properties: (1) hydrophobic: norleucine, met, ala, val, leu, ile; (2) neutral hydrophilic: cys, ser, thr; (3) acidic: asp, glu; (4) basic: asn, gln, his, lys, arg; (5) residues that influence chain orientation: gly, pro; and (6) aromatic : trp, tyr, phe.

[0075] . Non-conservative substitutions will entail exchanging a member of one of these classes for another class. Any cysteine residue not involved in maintaining the proper conformation of the bispecific binding molecule may be substituted, generally with serine, to improve the oxidative stability of the molecule and prevent aberrant crosslinking.

5 Conversely, cysteine bond (s) may be added to the antibody to improve its stability (particularly where the antibody is an antibody fragment such as an Fv fragment).

[0076] . A particularly preferred type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody (e. g. a humanized or human antibody). Generally, the resulting variant (s) selected for further development will have improved biological properties relative to the parent antibody from which they are generated. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Briefly, several hypervariable region sites (e. g. 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (e. g. binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the binding domain and, e.g., human 5T4. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with superior properties in one or more relevant assays may be selected for further development.

[0077] . Other modifications of the bispecific binding molecule are contemplated herein. For example, the bispecific binding molecule may be linked to one of a variety of nonproteinaceous polymers, e.g., polyethylene glycol, polypropylene glycol, polyoxyalkylenes, or copolymers of polyethylene glycol and polypropylene glycol. The bispecific binding molecule also may be entrapped in microcapsules prepared, for example, by coacervation techniques or by interfacial polymerization (for example, hydroxymethylcellulose or gelatin-microcapsules and poly (methylmethacrylate) microcapsules, respectively), in colloidal drug delivery systems (for example, liposomes, albumin microspheres, microemulsions, nano-particles and nanocapsules), or in macroemulsions. Such techniques are disclosed in Remington's Pharmaceutical Sciences, 16th edition, Oslo, A., Ed., (1980).

[0078] . The bispecific binding molecules disclosed herein may also be formulated as immunoliposomes. A "liposome" is a small vesicle composed of various types of lipids, phospholipids and/or surfactant which is useful for delivery of a drug to a mammal. The components of the liposome are commonly arranged in a bilayer formation, similar to the lipid arrangement of biological membranes. Liposomes containing the antibody are prepared by methods known in the art, such as described in Epstein et al., Proc. Natl. Acad. Sci. USA, 82: 3688 (1985); Hwang et al. , Proc. Natl Acad. Sci. USA, 77: 4030 (1980); U. S. Pat. Nos. 4, 485, 045 and 4,544, 545; and W097/38731 published October 23, 1997. Liposomes with enhanced circulation time are disclosed in U. S. Patent No. 5,013, 556. Particularly useful liposomes can be generated by the reverse phase evaporation method with a lipid composition comprising phosphatidylcholine, cholesterol and PEG-derivatized phosphatidylethanolamine (PEG-PE). Liposomes are extruded through filters of defined pore size to yield liposomes with the desired diameter. Fab' fragments of the antibody of the present invention can be conjugated to the liposomes as described in Martin et al. J. Biol. Chem. 257: 286-288 (1982) via a disulfide interchange reaction. A chemotherapeutic agent is optionally contained within the liposome. See Gabizon et al. J. National Cancer Inst. 81 (19) 1484 (1989).

[0079] . When using recombinant techniques, the bispecific binding molecule can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the bispecific binding molecule is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10: 163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of E coli.

[0080] . The bispecific binding molecule composition prepared from the cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique.

[0081] . In a further aspect, the present invention relates to a nucleic acid sequence encoding a bispecific binding molecule of the invention.

Said nucleic acid molecule is preferably comprised in a vector which is preferably comprised in a host cell. Said host cell is, e.g. after transformation or transfection with the nucleic acid sequence of the invention, capable of expressing the bispecific binding molecule. For that purpose the nucleic acid molecule is operatively linked with control sequences.

[0082] . As used herein, the term "host cell" is intended to refer to a cell into which a nucleic acid encoding the bispecific binding molecule of the invention is introduced by way of

transformation, transfection and the like. It should be understood that such terms refer not only to the particular subject cell but to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in fact, be identical to the parent cell, but
5 are still included within the scope of the term as used herein.

[0083] . As used herein, the term "expression" includes any step involved in the production of a bispecific binding molecule of the invention including, but not limited to, transcription, post-transcriptional modification, translation, post-translational modification, and secretion.

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

15 **[0084]** . Nucleic acid is "operably linked" when it is placed into a functional relationship with another nucleic acid sequence. For example, DNA for a presequence or secretory leader is operably linked to DNA for a polypeptide if it is expressed as a preprotein that participates in the secretion of the polypeptide; a promoter or enhancer is operably linked to a coding
20 sequence if it affects the transcription of the sequence; or a ribosome binding site is operably linked to a coding sequence if it is positioned so as to facilitate translation. Generally, "operably linked" means that the DNA sequences being linked are contiguous, and, in the case of a secretory leader, contiguous and in reading phase. However, enhancers do not have to be contiguous. Linking is accomplished by ligation at convenient restriction sites. If
25 such sites do not exist, the synthetic oligonucleotide adaptors or linkers are used in accordance with conventional practice.

[0085] . Suitable host cells include prokaryotes and eukaryotic host cells including yeasts, fungi, insect cells and mammalian cells.

30 **[0086]** . The bispecific binding molecule of the invention can be produced in bacteria. After expression, the bispecific binding molecule of the invention, preferably the bispecific binding molecule is isolated from the E. coli cell paste in a soluble fraction and can be purified through, e.g., affinity chromatography and/or size exclusion. Final purification can be carried out similar to the process for purifying antibody expressed e. g, in CHO cells.

35 In addition to prokaryotes, eukaryotic microbes such as filamentous fungi or yeast are suitable cloning or expression hosts for the bispecific binding molecule of the invention.

Saccharomyces cerevisiae, or common baker's yeast, is the most commonly used among lower eukaryotic host microorganisms. However, a number of other genera, species, and strains are commonly available and useful herein, such as Schizosaccharomyces pombe; Kluyveromyces hosts such as, e. g. , K. lactis, K. fragilis (ATCC 12424), K. bulgaricus (ATCC 16045), K. wickerhamii (ATCC 24178), K. waltii (ATCC 56500), K. drosophilae (ATCC 36906), K. thermotolerans, and K. marxianus; Yarrowia (EP 402 226); Pichia pastoris (EP 183 070); Candida; Trichoderma reesei (EP 244 234); Neurospora crassa; Schwanniomyces such as Schwanniomyces occidentalis; and filamentous fungi such as, e.g., Neurospora, Penicillium, Tolypocladium, and Aspergillus hosts such as A. nidulans and A. niger.

5 **[0087]** . Suitable host cells for the expression of glycosylated bispecific binding molecule of the invention, preferably antibody derived bispecific binding molecules are derived from multicellular organisms. Examples of invertebrate cells include plant and insect cells. Numerous baculoviral strains and variants and corresponding permissive insect host cells from hosts such as Spodoptera frugiperda (caterpillar), Aedes aegypti (mosquito), Aedes albopictus (mosquito), Drosophila melanogaster (fruit fly), and Bombyx mori have been identified. A variety of viral strains for transfection are publicly available, e. g. , the L-1 variant of Autographa californica NPV and the Bm-5 strain of Bombyx mori NPV, and such viruses may be used as the virus herein according to the present invention, particularly for transfection of Spodoptera frugiperda cells.

20 **[0088]** . Plant cell cultures of cotton, corn, potato, soybean, petunia, tomato, Arabidopsis and tobacco can also be utilized as hosts. Cloning and expression vectors useful in the production of proteins in plant cell culture are known to those of skill in the art. See e.g. Hiatt et al., Nature (1989) 342: 76-78, Owen et al. (1992) Bio/Technology 10: 790-794, Artsenko et al. (1995) The Plant J 8: 745-750, and Fecker et al. (1996) Plant Mol Biol 32: 979-986.

25 **[0089]** . However, interest has been greatest in vertebrate cells, and propagation of vertebrate cells in culture (tissue culture) has become a routine procedure. Examples of useful mammalian host cell lines are monkey kidney CV1 line transformed by SV40 (COS-7, ATCC CRL 1651) ; human embryonic kidney line (293 or 293 cells subcloned for growth in suspension culture, Graham et al. , J. Gen Virol. 36 : 59 (1977)); baby hamster kidney cells (BHK, ATCC CCL 10); Chinese hamster ovary cells/-DHFR (CHO, Urlaub et al. , Proc. Natl. Acad. Sci. USA 77: 4216 (1980)); mouse sertoli cells (TM4, Mather, Biol. Reprod. 23: 243-251 (1980)); monkey kidney cells (CV1 ATCC CCL 70); African green monkey kidney cells (VERO-76, ATCC CRL1587) ; human cervical carcinoma cells (HELA, ATCC CCL 2); canine kidney cells (MDCK, ATCC CCL 34); buffalo rat liver cells (BRL 3A, ATCC CRL 1442); human lung cells (W138, ATCC CCL 75); human liver cells (Hep G2, 1413 8065); mouse mammary tumor (MMT 060562, ATCC CCL5 1); TRI cells (Mather et al., Annals N. Y Acad. Sci. 383 : 44-68 (1982)) ; MRC 5 cells; FS4 cells; and a human hepatoma line (Hep G2).

[0090] . When using recombinant techniques, the bispecific binding molecule of the invention can be produced intracellularly, in the periplasmic space, or directly secreted into the medium. If the bispecific binding molecule is produced intracellularly, as a first step, the particulate debris, either host cells or lysed fragments, are removed, for example, by centrifugation or ultrafiltration. Carter et al., Bio/Technology 10: 163-167 (1992) describe a procedure for isolating antibodies which are secreted to the periplasmic space of E coli. Briefly, cell paste is thawed in the presence of sodium acetate (pH 3.5), EDTA, and phenylmethylsulfonylfluoride (PMSF) over about 30 min. Cell debris can be removed by centrifugation. Where the antibody is secreted into the medium, supernatants from such expression systems are generally first concentrated using a commercially available protein concentration filter, for example, an Amicon or Millipore Pellicon ultrafiltration unit. A protease inhibitor such as PMSF may be included in any of the foregoing steps to inhibit proteolysis and antibiotics may be included to prevent the growth of adventitious contaminants.

[0091] . The bispecific binding molecule of the invention prepared from the host cells can be purified using, for example, hydroxylapatite chromatography, gel electrophoresis, dialysis, and affinity chromatography, with affinity chromatography being the preferred purification technique.

[0092] . The matrix to which the affinity ligand is attached is most often agarose, but other matrices are available. Mechanically stable matrices such as controlled pore glass or poly(styrenedivinyl) benzene allow for faster flow rates and shorter processing times than can be achieved with agarose. Where the bispecific binding molecule of the invention comprises a CH3 domain, the Bakerbond ABXMresin (J. T. Baker, Phillipsburg, NJ) is useful for purification. Other techniques for protein purification such as fractionation on an ion-exchange column, ethanol precipitation, Reverse Phase HPLC, chromatography on silica, chromatography on heparin SEPHAROSETM chromatography on an anion or cation exchange resin (such as a polyaspartic acid column), chromato-focusing, SDS-PAGE, and ammonium sulfate precipitation are also available depending on the antibody to be recovered.

[0093] . In another aspect, processes are provided for producing bispecific binding molecules of the invention, said processes comprising culturing a host cell defined herein under conditions allowing the expression of the bispecific binding molecule and recovering the produced bispecific binding molecule from the culture.

[0094] . In an alternative embodiment, compositions are provided comprising a bispecific binding molecule of the invention, or produced according to the process of the invention. Preferably, said composition is a pharmaceutical composition.

[0095] . As used herein, the term “pharmaceutical composition” relates to a composition for administration to a patient, preferably a human patient. The particular preferred pharmaceutical composition of this invention comprises the bispecific binding molecule of the invention. Preferably, the pharmaceutical composition comprises suitable formulations of carriers, stabilizers and/or excipients. In a preferred embodiment, the 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, 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 bispecific binding molecule of the invention can be administered by using said pump systems. Such pump systems are generally known in the art, and commonly rely on periodic exchange of cartridges containing the therapeutic agent to be infused. When exchanging the cartridge in such a pump system, a temporary interruption of the otherwise uninterrupted flow of therapeutic agent into the body of the patient may ensue. In such a case, the phase of administration prior to cartridge replacement and the phase of administration following cartridge replacement would still be considered within the meaning of the pharmaceutical means and methods of the invention together make up one “uninterrupted administration” of such therapeutic agent.

[0096] . The continuous or uninterrupted administration of these bispecific binding molecules of the invention may be intravenous or subcutaneous by way of a fluid delivery device or small pump system including a fluid driving mechanism for driving fluid out of a reservoir and an actuating mechanism for actuating the driving mechanism. Pump systems for subcutaneous administration may include a needle or a cannula for penetrating the skin of a patient and delivering the suitable composition into the patient's body. Said pump systems may be directly fixed or attached to the skin of the patient independently of a vein, artery or blood vessel, thereby allowing a direct contact between the pump system and the skin of the patient. The pump system can be attached to the skin of the patient for 24 hours up to several days. The pump system may be of small size with a reservoir for small volumes. As a non-limiting example, the volume of the reservoir for the suitable pharmaceutical composition to be administered can be between 0.1 and 50 ml.

[0097] . 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.

[0098] . The inventive compositions 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. In general, as used herein, "pharmaceutically acceptable carrier" means any and all solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is well known in the art. Acceptable carriers, excipients, or stabilizers are nontoxic to recipients at the dosages and concentrations employed and include: additional buffering agents; preservatives; co-solvents; antioxidants, including ascorbic acid and methionine; chelating agents such as EDTA; metal complexes (e.g., Zn-protein complexes); biodegradable polymers, such as polyesters; salt-forming counterions, such as sodium, polyhydric sugar alcohols; amino acids, such as alanine, glycine, asparagine, 2-phenylalanine, and threonine; sugars or sugar alcohols, such as trehalose, sucrose, octasulfate, sorbitol or xylitol stachyose, mannose, sorbose, xylose, ribose, myoinisitose, galactose, lactitol, ribitol, myoinisitol, galactitol, glycerol, cyclitols (e.g., inositol), polyethylene glycol; sulfur containing reducing agents, such as glutathione, thiocetic acid, sodium thioglycolate, thioglycerol, [alpha]-monothioglycerol, and sodium thio sulfate; low molecular weight proteins, such as human serum albumin, bovine serum albumin, gelatin, or other immunoglobulins; and hydrophilic polymers, such as polyvinylpyrrolidone. 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 detergents are Tween 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.

[0099] . The compositions of the present invention can be administered to the subject at a
5 suitable dose which can be determined e.g. by dose escalating studies by administration of
increasing doses of the polypeptide of the invention exhibiting cross-species specificity
described herein to non-chimpanzee primates, for instance macaques. As set forth above,
the polypeptide of the invention exhibiting cross-species specificity described herein can be
advantageously used in identical form in preclinical testing in non-chimpanzee primates and
10 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 polypeptide of the invention as defined
herein or separately before or after administration of said polypeptide in timely defined
intervals and doses. The dosage regimen will be determined by the attending physician and
15 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.

[0100] . Preparations for parenteral administration include sterile aqueous or non-aqueous
20 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 vehicles include sodium
chloride solution, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed
25 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
30 origin. It is envisaged that the composition of the invention might comprise, in addition to the
polypeptide of the invention defined herein, further biologically active agents, depending on
the intended use of the composition. Such agents might be drugs acting on the gastro-
intestinal system, drugs acting as cytostatica, drugs preventing hyperurikemia, drugs
inhibiting immunoreactions (e.g. corticosteroids), drugs modulating the inflammatory
35 response, drugs acting on the circulatory system and/or agents such as cytokines known in
the art. It is also envisaged that the bispecific binding molecule of the present invention is
applied in a co-therapy, i.e., in combination with another anti-cancer medicament.

[0101] . The biological activity of the pharmaceutical composition defined herein can be determined for instance by cytotoxicity assays, as described in the following examples, in WO 99/54440 or by Schlereth et al. (Cancer Immunol. Immunother. 20 (2005), 1 – 12).

“Efficacy” or “in vivo efficacy” as used herein refers to the response to therapy by the

pharmaceutical composition of the invention, using e.g. standardized NCI response criteria.

The success or *in vivo* efficacy of the therapy using a pharmaceutical composition of the invention refers to the effectiveness of the composition for its intended purpose, i.e. the ability of the composition to cause its desired effect, i.e. depletion of pathologic cells, e.g.

tumor cells. The *in vivo* efficacy may be monitored by established standard methods for the

respective disease entities including, but not limited to white blood cell counts, differentials, Fluorescence Activated Cell Sorting, bone marrow aspiration. In addition, various disease specific clinical chemistry parameters and other established standard methods may be used.

Furthermore, computer-aided tomography, X-ray, nuclear magnetic resonance tomography (e.g. for National Cancer Institute-criteria based response assessment [Cheson BD, Horning

SJ, Coiffier B, Shipp MA, Fisher RI, Connors JM, Lister TA, Vose J, Grillo-Lopez A, Hagenbeek A, Cabanillas F, Klippensten D, Hiddemann W, Castellino R, Harris NL, Armitage JO, Carter W, Hoppe R, Canellos GP. Report of an international workshop to standardize

response criteria for non-Hodgkin's lymphomas. NCI Sponsored International Working Group. J Clin Oncol. 1999 Apr;17(4):1244]), positron-emission tomography scanning, white

blood cell counts, differentials, Fluorescence Activated Cell Sorting, bone marrow aspiration, lymph node biopsies/histologies, and various lymphoma specific clinical chemistry parameters (e.g. lactate dehydrogenase) and other established standard methods may be used.

[0102] . Another major challenge in the development of drugs such as the pharmaceutical

composition of the invention is the predictable modulation of pharmacokinetic properties. To this end, a pharmacokinetic profile of the drug candidate, i.e. a profile of the pharmacokinetic parameters that affect the ability of a particular drug to treat a given condition, can be

established. Pharmacokinetic parameters of the drug influencing the ability of a drug for treating a certain disease entity include, but are not limited to: half-life, volume of distribution,

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

[0103] . “Half-life” means the time where 50% of an administered drug are eliminated through biological processes, e.g. metabolism, excretion, etc.

[0104] . 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.

[0105] . "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.

[0106] . "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.

[0107] . Pharmacokinetic parameters also include bioavailability, lag time (T_{lag}), T_{max}, absorption rates, more onset and/or C_{max} 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.

[0108] . "T_{max}" is the time after which maximal blood concentration of the drug is reached, and "C_{max}" 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).

[0109] . 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.

[0110] . The term "safety", "in vivo safety" or "tolerability" as used herein defines the administration of a drug without inducing severe adverse events directly after administration (local tolerance) and during a longer period of application of the drug. "Safety", "in vivo safety" or "tolerability" can be evaluated e.g. at regular intervals during the treatment and follow-up period. Measurements include clinical evaluation, e.g. organ manifestations, and screening of laboratory abnormalities. Clinical evaluation may be carried out and deviations to normal findings recorded/coded according to NCI-CTC and/or MedDRA standards. Organ manifestations may include criteria such as allergy/immunology, blood/bone marrow, cardiac arrhythmia, coagulation and the like, as set forth e.g. in the Common Terminology Criteria for adverse events v3.0 (CTCAE). Laboratory parameters which may be tested include for instance 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 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.

5 **[0111]** . The term "effective dose" or "effective dosage" is defined as an amount sufficient to achieve or at least partially achieve the desired effect. The term "therapeutically effective dose" is defined as an amount sufficient to cure or at least partially arrest the disease and its complications in a patient already suffering from the disease. Amounts effective for this use will depend upon the severity of the infection and the general state of the subject's own
10 immune system. The term "patient" includes human and other mammalian subjects that receive either prophylactic or therapeutic treatment.

[0112] . The term "effective and non-toxic dose" as used herein refers to a tolerable dose of an inventive bispecific binding molecule which is high enough to cause depletion of pathologic cells, tumor elimination, tumor shrinkage or stabilization of disease without or
15 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).

[0113] . The above terms are also referred to e.g. in the Preclinical safety evaluation of biotechnology-derived pharmaceuticals S6; ICH Harmonised Tripartite Guideline; ICH
20 Steering Committee meeting on July 16, 1997.

[0114] . The appropriate dosage, or therapeutically effective amount, of the bispecific binding molecule of the invention will depend on the condition to be treated, the severity of the condition, prior therapy, and the patient's clinical history and response to the therapeutic
25 agent. The proper dose can be adjusted according to the judgment of the attending physician such that it can be administered to the patient one time or over a series of administrations. The pharmaceutical composition can be administered as a sole therapeutic or in combination with additional therapies such as anti-cancer therapies as needed.

[0115] . The pharmaceutical compositions of this invention are particularly useful for
30 parenteral administration, i.e., subcutaneously, intramuscularly, intravenously, intra-articular and/or intra-synovial. Parenteral administration can be by bolus injection or continuous infusion.

[0116] . If the pharmaceutical composition has been lyophilized, the lyophilized material is
35 first reconstituted in an appropriate liquid prior to administration. The lyophilized material may be reconstituted in, e.g., bacteriostatic water for injection (BWFI), physiological saline,

phosphate buffered saline (PBS), or the same formulation the protein had been in prior to lyophilization.

[0117] . Preferably, the bispecific binding molecule of the invention or produced by a process of the invention is used in the prevention, treatment or amelioration of a disease selected from a proliferative disease, a tumorous disease, or an immunological disorder.

[0118] . An alternative embodiment of the invention provides a method for the treatment or amelioration of a disease selected from a proliferative disease, a tumorous disease, or an immunological disorder comprising the step of administering to a patient in the need thereof the bispecific binding molecule of the invention or produced by a process of the invention.

[0119] . The formulations described herein are useful as pharmaceutical compositions in the treatment and/or prevention of the pathological medical condition as described herein in a patient in need thereof. The term "treatment" refers to both therapeutic treatment and prophylactic or preventative measures. Treatment includes the application or administration of the formulation to the body, an isolated tissue, or cell from a patient who has a disease/disorder, a symptom of a disease/disorder, or a predisposition toward a disease/disorder, with the purpose to cure, heal, alleviate, relieve, alter, remedy, ameliorate, improve, or affect the disease, the symptom of the disease, or the predisposition toward the disease.

[0120] . Those "in need of treatment" include those already with the disorder, as well as those in which the disorder is to be prevented. The term "disease" is any condition that would benefit from treatment with the protein formulation described herein. This includes chronic and acute disorders or diseases including those pathological conditions that predispose the mammal to the disease in question. Non-limiting examples of diseases/disorders to be treated herein include proliferative disease, a tumorous disease, or an immunological disorder.

[0121] . In another aspect, kits are provided comprising a bispecific binding molecule of the invention, a nucleic acid molecule of the invention, a vector of the invention, or a host cell of the invention. The kit may comprise one or more vials containing the bispecific binding molecule and instructions for use. The kit may also contain means for administering the bispecific binding molecule of the present invention such as a syringe, pump, infusor or the like.

[0122] . In still another aspect, the present invention relates to the use of amino acid residues 170 to 222 (epitope cluster 4) and/or 78 to 138 (epitope cluster 2) of the human 5T4 sequence as depicted in SEQ ID NO: 2 for the generation of an antibody, preferably a bispecific binding molecule having preferably the capacity to bind to human 5T4 and CD3 as described herein.

[0123] . Also, the present invention relates to a method for the generation of an antibody, preferably bispecific binding molecule, comprising (a) immunizing an animal with a protein comprising amino acid residues 170 to 222 (epitope cluster 4) and/or 78 to 138 (epitope cluster 2) of the human 5T4 sequence as depicted in SEQ ID NO: 2, (b) obtaining said antibody, and optionally (c) converting said antibody into a bispecific binding molecule having preferably the capacity to bind to human 5T4 and CD3 as described herein.

[0124] . The present invention further contemplates a fragment of 5T4 consisting of amino acid residues 170 to 222 (epitope cluster 4) or amino acids 78 to 138 (epitope cluster 2) of the human 5T4 sequence as depicted in SEQ ID NO: 2.

[0125] . It should be understood that the inventions herein are not limited to particular methodology, protocols, or reagents, as such can vary. The discussion and examples provided herein are presented for the purpose of describing particular embodiments only and are not intended to limit the scope of the present invention, which is defined solely by the claims.

[0126] . All publications and patents cited throughout the text of this specification (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether supra or infra, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention. To the extent the material incorporated by reference contradicts or is inconsistent with this specification, the specification will supersede any such material.

The Figures show:

[0127] . **Figure 1:** Hypothetical structure of 5T4. SRR: serine-rich repeat (brown); LRR: leucine-rich repeat (blue); cysteines are depicted by yellow circles; potential glycosylation sites are marked by asterisks.

[0128] . Figure 2: Structure model of 5T4 with highlighted segments exchanged in chimeric constructs as designed for epitope clustering. LLR: Leucin rich repeat. SRR: Serine rich repeat. Asterisks: position of carbohydrates.

5 **[0129] . Figure 3:** Epitope clustering with chimeric 5T4 constructs - Sequence alignment of human and murine 5T4.

Alignment of the amino acid sequences of human and murine 5T4. The intracellular and transmembrane domains are marked by a dotted box. Amino acid segments where the homologous murine sequence was substituted for the human sequence in the chimeric
10 constructs are also boxed and the amino acid positions referring to the human sequence are given.

[0130] . Figure 4: Murine and human 5T4 as well as seven chimeric human-murine 5T4 constructs expressed on the surface of CHO cells as shown by flow cytometry. The
15 expression of human 5T4 and the chimeric 5T4 molecules hu 5T4 **E1** mu, hu 5T4 **E2** mu, hu 5T4 **E3** mu and hu 5T4 **E6** mu on CHO was detected with a monoclonal anti-human 5T4 antibody. The expression of hu 5T4 **E4** mu, hu 5T4 **E5** mu and hu 5T4 **E7** mu was detected with a polyclonal anti-human 5T4 antibody. Murine 5T4 expression was detected with a polyclonal anti-mouse 5T4-antibody. Bound monoclonal antibody was detected with an anti-
20 mouse Fc-gamma-specific antibody conjugated to phycoerythrin. Bound polyclonal antibodies were detected with a PE-labeled anti-sheep antibody.

[0131] . Figure 5: Determination of binding constants of bispecific antibodies on human and macaque 5T4 and on human and macaque CD3 using the Biacore system. Antigen was
25 immobilized in low to intermediate density (100 RU) on CM5 chip. Dilutions of bispecific antibodies were floated over the chip surface and binding determined using BiaEval Software. Respective on- and off-rates and the resulting binding constant (KD) of the respective bispecific antibodies are depicted below every graph. Numbers in brackets indicate imprecise off-rate and KD calculation due to a biphasic off-rate of the respective 5T4
30 bispecific antibody.

[0132] . Figure 6: Determination of binding constants of bispecific antibodies directed against epitope 4 on 5T4 antigen positive cells. Cells were incubated with triplicate dilution series of respective bispecific antibodies. Detection was carried out in a strictly monovalent
35 manner using anti-His Fab/Alexa488 as detection antibody. 5T4 antigen positive cells used: NCI-N87 as naturally human 5T4 expressing cell line (left column), transfected CHO cells expressing recombinant 5T4 (middle column) and transfected CHO cells expressing

recombinant 5T4 (right column). Hyperbole graphs are shown with Scatchard analysis incorporated in every graph. Respective KD values are included in each diagram.

[0133] . Figure 7: Determination of binding constants of bispecific antibodies directed against epitope 7 on 5T4 antigen positive cells. Cells were incubated with triplicate dilution series of respective bispecific antibodies. Detection was carried out in a strictly monovalent manner using anti-His Fab/Alexa488 as detection antibody. 5T4 antigen positive cells used: NCI-N87 as naturally human 5T4 expressing cell line (left column), transfected CHO cells expressing recombinant 5T4 (middle column) and transfected CHO cells expressing recombinant 5T4 (right column). Hyperbole graphs are shown with Scatchard analysis incorporated in every graph. Respective KD values are included in each diagram.

[0134] . Figure 8: Determination of binding constants of bispecific antibodies directed against epitope E2/4 on 5T4 antigen positive cells. Cells were incubated with triplicate dilution series of respective bispecific antibodies. Detection was carried out in a strictly monovalent manner using anti-His Fab/Alexa488 as detection antibody. 5T4 antigen positive cells used: NCI-N87 as naturally human 5T4 expressing cell line (left column), transfected CHO cells expressing recombinant 5T4 (middle column) and transfected CHO cells expressing recombinant 5T4 (right column). Hyperbole graphs are shown with Scatchard analysis incorporated in every graph. Respective KD values are included in each diagram.

[0135] . Figure 9: Cytotoxic activity of 5T4 bispecific antibodies as measured in an 18-hour ⁵¹chromium release assay. Effector cells: stimulated enriched human CD8 T cells. Target cells: 5T4-positive human tumor cell line HCT116. Effector to target cell (E:T) ratio: 10:1. Ranking intervals based on current bispecific antibody experience for cytotoxic activity with stimulated enriched human CD8 T cells (EC50) <10 pg/ml (1), [10-100 pg/ml] (2), [100-1000 pg/ml] (3), [1000-5000 pg/ml] (4), ≥5000 pg/ml pg/ml (5).

[0136] . Figure 10: Cytotoxic activity of 5T4 bispecific antibodies as measured in a 48 hour FACS-based cytotoxicity assay. Effector cells: unstimulated human PBMC. Target cells: CHO cells transfected with human 5T4. Effector to target cell (E:T)-ratio: 10:1. Ranking intervals based on current bispecific antibody experience for cytotoxic activity with unstimulated human PBMC (EC50): <250 pg/ml (1), [250-1000 pg/ml] (2), [1000-5000 pg/ml] (3), [5000-20000 pg/ml] (4), ≥20000 pg/ml pg/ml (5).

[0137] . Figure 11: Cytotoxic activity of 5T4 bispecific antibodies as measured in a 48 hour FACS-based cytotoxicity assay. Effector cells: unstimulated human PBMC. Target cells: 5T4-positive human tumor cell line NCI-N87. Effector to target cell (E:T)-ratio: 10:1. Ranking intervals based on current bispecific antibody experience for cytotoxic activity with unstimulated human PBMC (EC50): <250 pg/ml (1), [250-1000 pg/ml] (2), [1000-5000 pg/ml] (3), [5000-20000 pg/ml] (4), ≥ 20000 pg/ml (5).

[0138] . Figure 12: Cytotoxic activity of 5T4 bispecific antibodies as measured in a 48 hour FACS-based cytotoxicity assay. Effector cells: macaque T cell line 4119LnPx. Target cells: CHO cells transfected with macaque 5T4. Effector to target cell (E:T) ratio: 10:1. Ranking intervals based on current bispecific antibody experience for cytotoxic activity with the macaque T cell line 4119LnPx (EC50): <250 pg/ml (1), [250-1000 pg/ml] (2), [1000-5000 pg/ml] (3), [5000-20000 pg/ml] (4), ≥ 20000 pg/ml (5).

[0139] . Figure 13: Anti-tumor activity of bispecific antibodies in a HCT-116 advanced stage tumor model

HCT-116 human colon carcinoma cells were subcutaneously injected into the right dorsal flank of NOD/SCID mice (n=10 per group). After tumors had reached a volume of approximately 180-200 mm³, human T cells were transplanted into the peritoneal cavity. A 5T4-CD3 bispecific antibody (5T4-12 HL x CD3 HL) (0.1, 0.5 and 2.5 mg/kg/d) was administered by intravenous bolus injection into the lateral tail vein for 14 days. Control animals (Group 2) and animals without T cell injection (Group 1) received the vehicle by intravenous bolus injection into the lateral tail vein for 14 days. Growth of tumors was determined by external caliper measurements, and tumor volumes were calculated using a standard hemi-ellipsoid formula: (length [mm] x width [mm]²)/2.

[0140] . Figure 14: Anti-tumor activity of bispecific antibodies in a in a NCI-N87 advanced stage tumor model

NCI-N87 human gastric carcinoma cells were subcutaneously injected into the right dorsal flank of NOD/SCID mice (n=10 per group). After tumors had reached a volume of approximately 180-200 mm³, human T cells were transplanted into the peritoneal cavity. A 5T4-CD3 bispecific antibody (5T4-12 HL x CD3 HL) (0.1, 0.5 and 2.5 mg/kg/d) was administered by intravenous bolus injection into the lateral tail vein for 21 days. Control animals (Group 2) and animals without T cell injection (Group 1) received the vehicle by intravenous bolus injection into the lateral tail vein for 21 days. Growth of tumors was

determined by external caliper measurements, and tumor volumes were calculated using a standard hemi-ellipsoid formula: (length [mm] x width [mm]²)/2.

[0141] . Examples

- 5 The following examples illustrate the invention. These examples should not be construed as to limit the scope of this invention. The examples are included for purposes of illustration and the present invention is limited only by the claims.

[0142] . Example 1

10 **Generation of CHO cells expressing chimeric transmembrane 5T4**

For construction of the chimeric epitope mapping molecules, the amino acid sequence of the respective epitope domains of human 5T4 was changed to the murine sequence.

The following molecules were constructed:

- hu 5T4 E1 mu (amino acid 34-77: murine)
- 15 • hu 5T4 E2 mu (amino acid 78-138: murine)
- hu 5T4 E3 mu (amino acid 139-169: murine)
- hu 5T4 E4 mu (amino acid 170-222: murine)
- hu 5T4 E5 mu (amino acid 223-270: murine)
- hu 5T4 E6 mu (amino acid 271-299 murine)
- 20 • hu 5T4 E7 mu (amino acid 300-360 murine)

The cDNA constructs were cloned into the mammalian expression vector pEF-DHFR and stably transfected into CHO cells. The expression of human 5T4 and chimeric 5T4 molecules hu 5T4 E1 mu, hu 5T4 E2 mu, hu 5T4 E3 mu and hu 5T4 E6 mu on CHO was verified in a
25 FACS assay using a monoclonal anti-human 5T4 antibody (R&D Systems #MAB49751). The expression of hu 5T4 E4 mu, hu 5T4 E5 mu and hu 5T4 E7 mu was detected with a polyclonal anti-human 5T4 antibody (R&D Systems #AF4975). Mu 5T4 expression was demonstrated with a polyclonal anti-mouse 5T4-antibody (R&D Systems #AF5049). The used concentration of the 5T4 antibodies was 5 µg/ml. Bound monoclonal antibodies were
30 detected with an anti-mouse Fc gamma-specific antibody conjugated to R-phycoerythrin (1:100; Dianova #115-116-071). Bound polyclonal antibodies were detected with 5 µg/ml of a PE-labeled anti-sheep antibody (Santa Cruz Biotechnology #sc-3746). All antibodies were diluted in PBS with 2% FCS. As negative control, cells were incubated with PBS/2% FCS instead of the first antibody. The samples were measured by flow cytometry on a
35 FACSCanto II instrument (Becton Dickinson) and analyzed by FlowJo software (Version 7.6). The surface expression of human-murine 5T4 chimeras, transfected CHO cells were analyzed and confirm in a flow cytometry assay with different anti-5T4 antibodies (Figure 4).

[0143] . Example 2**2.1 Transient expression in HEK 293 cells**

Clones of the expression plasmids with sequence-verified nucleotide sequences were used for transfection and protein expression in the FreeStyle 293 Expression System (Invitrogen GmbH, Karlsruhe, Germany) according to the manufacturer's protocol. Supernatants containing the expressed proteins were obtained, cells were removed by centrifugation and the supernatants were stored at -20 C.

2.2 Stable expression in CHO cells

Clones of the expression plasmids with sequence-verified nucleotide sequences were transfected into DHFR deficient CHO cells for eukaryotic expression of the constructs. Eukaryotic protein expression in DHFR deficient CHO cells was performed as described by Kaufman R.J. (1990) Methods Enzymol. 185, 537-566. Gene amplification of the constructs was induced by increasing concentrations of methotrexate (MTX) to a final concentration of 20 nM MTX. After two passages of stationary culture the cells were 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 were removed by centrifugation and the supernatant containing the expressed protein was stored at -20 C.

[0144] . Example 3**Epitope clustering of murine scFv-fragments**

Cells transfected with human or murine 5T4, or with chimeric 5T4 molecules were stained with crude, undiluted periplasmic extract containing scFv binding to human/maaque 5T4. Bound scFv were detected with 1 µg/ml of an anti-FLAG antibody (Sigma F1804) and a R-PE-labeled anti-mouse Fc gamma-specific antibody (1:100; Dianova #115-116-071). All antibodies were diluted in PBS with 2% FCS. As negative control, cells were incubated with PBS/2% FCS instead of the periplasmic extract. The samples were measured by flow cytometry on a FACSCanto II instrument (Becton Dickinson) and analyzed by FlowJo software (Version 7.6).

[0145] . Example 4**Procurement of different recombinant forms of soluble human and macaque 5T4**

The coding sequences of human and macaque 5T4 (human and mouse 5T4 sequences as published in GenBank, accession numbers NM_006670 [human]; NM_011627 [mouse]) coding sequences of human albumin, murine Fcγ1 and murine albumin were used for the construction of artificial cDNA sequences encoding soluble fusion proteins of human and macaque 5T4 respectively and human albumin, murine IgG1 Fc and murine albumin respectively as well as soluble proteins comprising only the extracellular domains of 5T4. To

generate the constructs for expression of the soluble human and macaque 5T4 proteins, cDNA fragments were obtained by PCR mutagenesis of the full-length 5T4 cDNAs described above and molecular cloning according to standard protocols.

For the fusions with human albumin, the modified cDNA fragments were designed as to contain first a Kozak site for eukaryotic expression of the constructs followed by the coding sequence of the human and macaque 5T4 proteins respectively comprising the amino acids 1 to 355 corresponding to the signal peptide and extracellular domain of human and macaque 5T4 respectively, followed in frame by the coding sequence of an artificial Ser1-Gly4-Ser1-linker, followed in frame by the coding sequence of human serum albumin, followed in frame by the coding sequence of a Flag tag, followed in frame by the coding sequence of a modified histidine tag (SGHHGGHHGGHH) and a stop codon.

For the fusions with murine IgG1, the modified cDNA fragments were designed as to contain first a Kozak site for eukaryotic expression of the constructs followed by the coding sequence of the human and macaque 5T4 proteins respectively comprising the amino acids 1 to 355 corresponding to the signal peptide and extracellular domain of human and macaque 5T4 respectively, followed in frame by the coding sequence of an artificial Ser1-Gly4-Ser1-linker, followed in frame by the coding sequence of the hinge and Fc gamma portion of murine IgG1, followed in frame by the coding sequence of a hexahistidine tag and a stop codon.

For the fusions with murine albumin, the modified cDNA fragments were designed as to contain first a Kozak site for eukaryotic expression of the constructs followed by the coding sequence of the human and macaque 5T4 proteins respectively comprising the amino acids 1 to 355 corresponding to the signal peptide and extracellular domain of human and macaque 5T4 respectively, followed in frame by the coding sequence of an artificial Ser1-Gly4-Ser1-linker, followed in frame by the coding sequence of murine serum albumin, followed in frame by the coding sequence of a Flag tag, followed in frame by the coding sequence of a modified histidine tag (SGHHGGHHGGHH) and a stop codon.

For the soluble extracellular domain constructs, the modified cDNA fragments were designed as to contain first a Kozak site for eukaryotic expression of the constructs followed by the coding sequence of the human and macaque 5T4 proteins respectively comprising the amino acids 1 to 355 corresponding to the signal peptide and extracellular domain of human and macaque 5T4 respectively, followed in frame by the coding sequence of an artificial Ser1-Gly1-linker, followed in frame by the coding sequence of a Flag tag, followed in frame by the coding sequence of a modified histidine tag (SGHHGGHHGGHH) and a stop codon.

The cDNA fragments were also designed 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. The cDNA 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). The aforementioned procedures were 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)).

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[0146] . Example 5**Biacore-based determination of bispecific antibody affinity to human and macaque 5T4 and CD3**

Biacore analysis experiments were performed using recombinant 5T4 fusion proteins with human serum albumin (ALB) to determine 5T4 target binding. For CD3 affinity measurements, recombinant fusion proteins having the N-terminal 27 amino acids of the CD3 epsilon (CD3e) fused to human antibody Fc portion were used. This recombinant protein exists in a human CD3e1-27 version and in a cynomolgous CD3e version, both bearing the epitope of the CD3 binder in the bispecific antibodies.

In detail, CM5 Sensor Chips (GE Healthcare) were immobilized with approximately 100 to 150 RU of the respective recombinant antigen using acetate buffer pH4.5 according to the manufacturer's manual. The bispecific antibody samples were loaded in five concentrations: 50 nM, 25 nM, 12.5 nM, 6.25 nM and 3.13 nM diluted in HBS-EP running buffer (GE Healthcare). Flow rate was 30 to 35 ul/min for 3 min, then HBS-EP running buffer was applied for 8 min again at a flow rate of 30 to 35 ul/ml. Regeneration of the chip was performed using 10 mM glycine 0.5 M NaCl pH 2.45. Data sets were analyzed using BiaEval Software (Figure 5). In general two independent experiments were performed.

[0147] . Example 6**25 Bispecific binding and interspecies cross-reactivity**

For flow cytometry, 200,000 cells of the respective cell lines were incubated for 30 min on ice with 50 µl of purified bispecific molecules at a concentration of 5 µg/ml. The cells were washed twice in PBS with 2% FCS and binding of the constructs was detected with a murine PentaHis antibody (Qiagen; diluted 1:20 in 50 µl PBS with 2% FCS). After washing, bound PentaHis antibodies were detected with an Fc gamma-specific antibody (Dianova) conjugated to phycoerythrin, diluted 1:100 in PBS with 2% FCS.

[0148] . Example 7**35 Scatchard-based determination of bispecific-antibody affinity to human and macaque 5T4**

For Scatchard analysis, saturation binding experiments were performed using a monovalent detection system developed at Micromet (anti-His Fab/Alexa 488) to precisely determine monovalent binding of the bispecific antibodies to the respective cell line.

2 x 10⁴ cells of the respective cell line (natively 5T4 expressing human cancer cell line NCI-N87, recombinantly human 5T4-expressing CHO cell line, recombinantly macaque 5T4-expressing CHO cell line) were incubated with each 50 μ l of a triplet dilution series (eight dilutions at 1:2) of the respective 5T4 bispecific antibody starting at 100 nM followed by 16 h incubation at 4 °C under agitation and one residual washing step. Then, the cells were incubated for further 30 min with 30 μ l of an anti-His Fab/Alexa488 solution (Micromet; 30 μ g/ml). After one washing step, the cells were resuspended in 150 μ l FACS buffer containing 3.5 % formaldehyde, incubated for further 15 min, centrifuged, resuspended in FACS buffer and analyzed using a FACS Cantoll machine and FACS Diva software. Data were generated from two independent sets of experiments. Values were plotted as hyperbole binding curves. Respective Scatchard analysis was calculated to extrapolate maximal binding (Bmax). The concentrations of bispecific antibodies at half-maximal binding were determined reflecting the respective KDs. Values of triplicate measurements were plotted as hyperbolic curves. Maximal binding was determined using Scatchard evaluation and the respective KDs were calculated. Values depicted in table 2 were derived from two independent experiments per 5T4 bispecific antibody. One representative experiment per 5T4 bispecific antibody is shown in Figures 6 - 8.

5T4 bispecific antibody	<i>KD [nM]</i>	<i>KD [nM]</i>	<i>KD [nM]</i>
	<i>Native human 5T4 NCI- N87 cells</i>	<i>Human 5T4 CHO cells</i>	<i>Macaque 5T4 CHO cells</i>
Epitope cluster 4			
5T4-CD3-1	2.54 $\pm$ 0.18	9.92 $\pm$ 6.18	18.68 $\pm$ 9.33
5T4-CD3-2	1.90 $\pm$ 0.98	5.50 $\pm$ 0.22	30.29 $\pm$ 4.63
5T4-CD3-3	3.70 $\pm$ 1.03	5.70 $\pm$ 0.11	35.39 $\pm$ 18.26
5T4-CD3-4	1.57 $\pm$ 0.19	5.49 $\pm$ 0.38	5.65 $\pm$ 1.25
Epitope cluster 7			
5T4-CD3-5	0.90 $\pm$ 0.40	0.72 $\pm$ 0.06	2.07 $\pm$ 0.09
5T4-CD3-6	0.67 $\pm$ 0.29	0.89 $\pm$ 0.03	2.08 $\pm$ 0.37
5T4-CD3-7	0.75 $\pm$ 0.15	1.01 $\pm$ 0.34	1.44 $\pm$ 0.33

Epitope cluster 2/4			
5T4-CD3-8	0.14 ± 0.07	0.29 ± 0.03	1.75 ± 0.85
5T4-CD3-9	0.25 ± 0.09	1.24 ± 0.52	2.79 ± 0.92
5T4-CD3-10	0.34 ± 0.23	0.17 ± 0.01	0.54 ± 0.26
5T4-CD3-11	0.13 ± 0.14	0.29 ± 0.03	1.75 ± 0.85
5T4-CD3-12	0.43 ± 0.11	0.94 ± 0.04	0.92 ± 0.06
5T4-CD3-13	0.08 ± 0.01	0.69 ± 0.16	3.35 ± 1.05
5T4-CD3-14	0.31 ± 0.18	0.65 ± 0.03	1.75 ± 0.52
5T4-CD3-15	0.39 ± 0.11	1.58 ± 0.23	3.34 ± 1.13

Table 2: Affinities (KD) of 5T4 bispecific antibodies from cell based Scatchard analysis (two independent experiments each)

5 The high affinities of the 15 5T4 bispecific antibodies measured by Biacore on recombinant soluble human and macaque 5T4-antigen could be confirmed by Scatchard analysis on CHO cells transfected with human or macaque 5T4. Most importantly, the affinities to native 5T4 expressed on the surface of the human tumor cell line NCI-N87 closely resemble those measured on CHO cells expressing recombinant 5T4 on their surface. All 5T4 bispecific antibodies of the E2/4 epitope cluster and all 5T4 bispecific antibodies of the E7 epitope cluster bind to native human 5T4 on NCI-N87 cells with sub-nM affinity, while four out of four 5T4 bispecific antibodies of the E4 epitope cluster bind to native human 5T4 with 1-digit nM affinity.

15 **[0149] . Example 8**

Cytotoxic activity

8.1 Chromium release assay with stimulated human T cells

Stimulated T cells enriched for CD8⁺ T cells were obtained as described below.

A petri dish (145 mm diameter, Greiner bio-one GmbH, Kremsmünster) was coated with a commercially available anti-CD3 specific antibody (OKT3, Orthoclone) in a final concentration of 1 µg/ml for 1 hour at 37°C. Unbound protein was removed by one washing step with PBS. 3 - 5 x 10⁷ human 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.

CD8⁺ cytotoxic T lymphocytes (CTLs) were enriched by depletion of CD4⁺ T cells and CD56⁺ NK cells using Dynal-Beads according to the manufacturer's protocol.

Macaque or human 5T4-transfected CHO target cells were 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 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 200 μl supplemented RPMI (as above) with an E:T ratio of 10:1. A starting concentration of 0.01 – 1 $\mu\text{g/ml}$ of purified bispecific antibody and threefold dilutions thereof were used. Incubation time for the assay was 18 hours. Cytotoxicity was determined as relative values of released chromium in the supernatant relative 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 chromium activity in the supernatants was performed in a Wizard 3" gammacounter (Perkin Elmer Life Sciences GmbH, Köln, Germany). Analysis of the results was carried out with Prism 5 for Windows (version 5.0, GraphPad Software Inc., San Diego, California, USA). EC50 values calculated by the analysis program from the sigmoidal dose response curves were used for comparison of cytotoxic activity.

8.2 FACS-based cytotoxicity assay with unstimulated human PBMC

Isolation of effector cells

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 NH_4Cl , 10 mM KHCO_3 , 100 μM EDTA). Platelets were removed via the supernatant upon centrifugation of PBMC at 100 x g. Remaining lymphocytes mainly encompass B and T lymphocytes, NK cells and monocytes. PBMC were kept in culture at 37°C/5% CO_2 in RPMI medium (Gibco) with 10% FCS (Gibco).

Depletion of CD14^+ and CD56^+ cells

For depletion of CD14^+ cells, human CD14 MicroBeads (Milteny Biotec, MACS, #130-050-201) were used, for depletion of NK cells human CD56 MicroBeads (MACS, #130-050-401). PBMC were counted and centrifuged for 10 min at room temperature with 300 x g. The supernatant was discarded and the cell pellet resuspended in MACS isolation buffer [80 $\mu\text{L}/10^7$ cells; PBS (Invitrogen, #20012-043), 0.5% (v/v) FBS (Gibco, #10270-106), 2 mM EDTA (Sigma-Aldrich, #E-6511)]. CD14 MicroBeads and CD56 MicroBeads (20 $\mu\text{L}/10^7$ cells) were added and incubated for 15 min at 4 - 8°C. The cells were washed with MACS isolation buffer (1 - 2 $\text{mL}/10^7$ cells). After centrifugation (see above), supernatant was discarded and cells resuspended in MACS isolation buffer (500 $\mu\text{L}/10^8$ cells). CD14/CD56 negative cells

were then isolated using LS Columns (Miltenyi Biotec, #130-042-401). PBMC w/o CD14+/CD56+ cells were cultured in RPMI complete medium i.e. RPMI1640 (Biochrom AG, #FG1215) supplemented with 10% FBS (Biochrom AG, #S0115), 1x non essential amino acids (Biochrom AG, #K0293), 10 mM Hepes buffer (Biochrom AG, #L1613), 1 mM sodium pyruvate (Biochrom AG, #L0473) and 100 U/mL penicillin/streptomycin (Biochrom AG, #A2213) at 37°C in an incubator until needed.

Target cell labeling

For the analysis of cell lysis in flow cytometry assays, the fluorescent membrane dye DiOC₁₈ (DiO) (Molecular Probes, #V22886) was used to label human or macaque 5T4-transfected CHO cells or 5T4-expressing human NCI-N87 cells as target cells and distinguish them from effector cells. Briefly, cells were harvested, washed once with PBS and adjusted to 10⁶ cell/mL in PBS containing 2 % (v/v) FBS and the membrane dye DiO (5 µL/10⁶ cells). After incubation for 3 min at 37°C, cells were washed twice in complete RPMI medium and the cell number adjusted to 1.25 x 10⁵ cells/mL. The vitality of cells was determined using 0.5 % (v/v) isotonic EosinG solution (Roth, #45380).

Flow cytometry based analysis

This assay was designed to quantify the lysis of macaque or human 5T4-transfected CHO cells in the presence of serial dilutions of 5T4 bispecific antibodies.

Equal volumes of DiO-labeled target cells and effector cells (i.e., PBMC w/o CD14⁺ cells) were mixed, resulting in an E:T cell ratio of 10:1. 160 µL of this suspension were transferred to each well of a 96-well plate. 40 µL of serial dilutions of the 5T4 bispecific antibodies and a negative control bispecific (an CD3-based bispecific antibody recognizing an irrelevant target antigen) or RPMI complete medium as an additional negative control were added. The bispecific antibody-mediated cytotoxic reaction proceeded for 48 hours in a 7% CO₂ humidified incubator. Then cells were transferred to a new 96-well plate and loss of target cell membrane integrity was monitored by adding propidium iodide (PI) at a final concentration of 1 µg/mL. PI is a membrane impermeable dye that normally is excluded from viable cells, whereas dead cells take it up and become identifiable by fluorescent emission.

Samples were measured by flow cytometry on a FACSCanto II instrument and analyzed by FACSDiva software (both from Becton Dickinson).

Target cells were identified as DiO-positive cells. PI-negative target cells were classified as living target cells. Percentage of cytotoxicity was calculated according to the following formula:

$$\text{Cytotoxicity} [\%] = \frac{n_{\text{dead target cells}}}{n_{\text{target cells}}} \times 100$$

n = number of events

Using GraphPad Prism 5 software (Graph Pad Software, San Diego), the percentage of cytotoxicity was plotted against the corresponding bispecific antibody concentrations. Dose response curves were analyzed with the four parametric logistic regression models for evaluation of sigmoid dose response curves with fixed hill slope and EC50 values were
5 calculated.

8.3 FACS-based cytotoxicity assay with a macaque T cell line

The macaque T cell line 4119LnPx (Knappe et al. Blood 95:3256-61 (2000), kindly provided by Prof Fickenscher, Hygiene Institute, Virology, Erlangen-Nuernberg) was used as source of
10 effector cells. Target cell labeling of macaque 5T4-transfected CHO cells and flow cytometry based analysis of cytotoxic activity was performed as described above.

[0150] . Example 9

Cytotoxic activity

15 The potency of human-like 5T4 bispecific antibodies in redirecting effector T cells against 5T4-expressing target cells was analyzed in five additional in vitro cytotoxicity assays:

1. The potency of 5T4 bispecific antibodies in redirecting stimulated human effector T cells against the 5T4-positive human tumor cell line HCT116 was measured in a 51-chromium release assay.
- 20 2. The potency of 5T4 bispecific antibodies in redirecting the T cells in unstimulated human PBMC against human 5T4-transfected CHO cells was measured in a FACS-based cytotoxicity assay.
3. The potency of 5T4 bispecific antibodies in redirecting the T cells in unstimulated human PBMC against the 5T4-positive human tumor cell line N87 was measured in a
25 FACS-based cytotoxicity assay.
4. For confirmation that the cross-reactive 5T4 bispecific antibodies are capable of redirecting macaque T cells against macaque 5T4-transfected CHO cells, a FACS-based cytotoxicity assay was performed with a macaque T cell line as effector T cells.
5. The potency gap between monomeric and dimeric forms of 5T4 bispecific antibodies
30 was determined in a 51-chromium release assay using human 5T4-transfected CHO cells as target cells and stimulated human T cells as effector cells.

9.1 Stimulated human T cells against the 5T4-positive human tumor cell line HCT116

The cytotoxic activity of 5T4 bispecific antibodies was analyzed in a 51-chromium (⁵¹Cr)
35 release cytotoxicity assay using the 5T4-positive human tumor cell line HCT116 (ATCC No

CCL-247) as source of target cells, and stimulated, enriched human CD8 T cells as effector cells (Figure 9).

In accordance with the results of the ⁵¹chromium release assays with stimulated enriched human CD8 T lymphocytes as effector cells and human 5T4-transfected CHO cells as targets, the most cytotoxic 5T4 bispecific antibodies with EC50 values as low as 1-digit pg/ml were found within the E2/4 epitope cluster when stimulated enriched human CD8 T lymphocytes were used as effector cells and the 5T4-positive human tumor cell line HCT116 served as target. Strong activity of 5T4 bispecific antibodies for the E4 epitope cluster could also be seen with EC50 values in the HCT116 assay of 2-digit pg/ml. 5T4 bispecific antibodies of the E7 epitope cluster, however, showed poor cytotoxicity against HCT116 cells with 2-digit ng/ml EC50 values, although their EC50 values for cytotoxic activity against human 5T4-transfected CHO cells was still within an acceptable 3-digit pg/ml range.

9.2 Unstimulated human PBMC against human 5T4-transfected target cells

The cytotoxic activity of 5T4 bispecific antibodies was also analyzed in a FACS-based cytotoxicity assay using CHO cells transfected with human 5T4 as target cells, and unstimulated human PBMC as effector cells (Figure 10).

The results of the FACS-based cytotoxicity assays with unstimulated human PBMC as effector cells and human 5T4-transfected CHO cells as targets confirmed the epitope-activity relationship already observed in the ⁵¹chromium release assays. Within the E2/4 epitope cluster six out of eight 5T4 bispecific antibodies proved to be very potent with 1-digit pg/ml EC50 values and two out of eight still presented with 2-digit pg/ml EC50 values. All four E4 cluster bispecifics were in the 2-digit pg/ml EC50 range, while the 5T4 bispecific antibodies of the E7 cluster required 3-digit pg/ml concentrations (i.e. EC50) for inducing half-maximal lysis of human 5T4-transfected CHO cells by unstimulated human PBMC.

9.3 Unstimulated human PBMC against the 5T4-positive human tumor cell line NCI-N87

The cytotoxic activity of 5T4 bispecific antibodies was furthermore analyzed in a FACS-based cytotoxicity assay using the 5T4-positive human tumor cell line NCI-N87 as source of target cells and unstimulated human PBMC as effector cells (Figure 11)

The results of the FACS-based cytotoxicity assays with unstimulated human PBMC as effector cells and the 5T4-positive human tumor cell line NCI-N87 as target again confirmed the superior cytotoxic activity of 5T4 bispecific antibodies mapped to the E2/4 cluster. In this assay the four 5T4 bispecific antibodies of the E4 epitope cluster proved to be almost as active as their E2/4 counterparts. However, the E7 cluster bispecific antibodies required 1-digit ng/ml concentrations (i.e. EC50) for inducing half-maximal lysis of human NCI-N87 tumor cells, which is in line with their poor cytotoxic activity against the other 5T4-positive

human tumor cell line (HCT116) as tested in the ⁵¹chromium release assays with stimulated enriched human CD8 effector T cells.

9.4 Macaque T cells against macaque 5T4-expressing target cells

Finally, the cytotoxic activity of 5T4 bispecific antibodies was analyzed in a FACS-based cytotoxicity assay using CHO cells transfected with macaque 5T4 as target cells, and a
5 macaque T cell line as source of effector cells (Figure 12).

The results of the FACS-based cytotoxicity assays in the macaque system closely resembled the finding in the human system. Accordingly, macaque T cells from cell line 4119LnPx were induced to efficiently kill macaque 5T4-transfected CHO cells by 5T4 bispecific antibodies of
10 the E2/4 cluster with 1-digit pg/ml EC50-value, by 5T4 bispecific antibodies of the E4 cluster with 2-digit pg/ml EC50-value and by 5T4 bispecific antibodies of the E7 cluster with 3- to 4-digit pg/ml EC50-values.

[0151] . Example 10

15 **Therapeutic efficacy of bispecific antibodies in human tumor xenograft models**

The human cancer cell lines HCT-116 and NCI-N87 were subcutaneously injected in the right dorsal flank of NOD.CB17-Prkdcscid/J mice.

After tumors had reached a volume 180-200 mm³, mice were randomized into treatment groups. In vitro expanded human T cells were transplanted into the mice by injection into the
20 peritoneal cavity of animals of groups. Mice of Group 1 (n=5) did not receive effector cells and were used as an untransplanted control for comparison with group 2 to monitor the impact of T cells alone on tumor growth.

The treatment started when the mean tumor volume had reached ~240 (HCT-116) and 200 mm³ (NCI-N87), respectively. The mean tumor size of each treatment group on the day of
25 treatment start was not statistically different from any other group (analysis of variance). Mice were treated with 0.1, 0.5 and 2.5 mg/kg/day of 5T4-12-CD3 by intravenous bolus injection for 14 (HCT-116) and 21 (NCI-N87) days, respectively.

Tumors were measured by caliper during the study and progress evaluated by intergroup comparison of tumor volumes.

Group	D15	D16	D17	D18	D19	D22	D24
1	1 [0]	1.09 [0.03]	1.17 [0.04]	1.28 [0.09]	1.42 [0.09]	1.71 [0.09]	2.03 [0.15]
2	1 [0]	1.12 [0.07]	1.24 [0.08]	1.44 [0.12]	1.63 [0.11]	1.94 [0.19]	2.40 [0.25]
3	1 [0]	0.87 [0.07]	0.74 [0.11]	0.69 [0.10]	0.52 [0.11]	0.59 [0.19]	0.60 [0.26]
4	1 [0]	0.89 [0.1]	0.79 [0.14]	0.74 [0.15]	0.67 [0.11]	0.60 [0.15]	0.60 [0.2]
5	1 [0]	0.93 [0.07]	0.85 [0.1]	0.78 [0.11]	0.69 [0.12]	0.62 [0.11]	0.59 [0.11]

Table 3 Relative tumor Volume in an HCT-116 advanced stage tumor model

The tumor volume at day x is expressed as Relative Tumor Volume (RTV) and calculated according to the following formula: $RTV = TV_x / TV_{15}$, where TV_x is the tumor volume at day x and TV_{15} is the tumor volume before the start of treatment on Day 15.

Group	Data	D15	D16	D17	D18	D19	D22	D24
Gr2 Vehicle	Median	236.95	270.72	295.87	331.65	382.51	470.5	568.21
	T/C (%)	100	100	100	100	100	100	100
Gr1 Vehicle w/o T cells	Median	228.93	259.52	276.71	297.01	334.00	401.21	495.27
	T/C (%)	97	96	94	90	87	85	87
Gr3 MTR118 2.5 mg/kg	Median	236.86	218.41	190.93	172.28	155.01	127.06	115.93
	T/C (%)	100	81	65	52	41	27	20
Gr4 MTR118 0.5 mg/kg	Median	236.4	222.54	191.7	181.24	166.52	141.17	134.06
	T/C (%)	100	82	65	55	44	30	24
Gr5 MTR118 0.1 mg/kg	Median	237.59	221.76	203.67	182.67	161.29	149.68	137.05
	T/C (%)	100	82	69	55	42	32	24

Table 4 bispecific antibody-mediated tumor growth inhibition in a HCT-116 advanced stage tumor model

The T/C% is determined by calculating TV as $T/C\% = 100 \times (\text{median TV of treated group}) / (\text{median TV of control group})$.

Group	D22	D23	D24	D25	D26	D29	D30	D31	D33	D36	D38	D40	D43
1	1 [0]	1.19 [0.23]	1.39 [0.16]	1.43 [0.13]	1.54 [0.21]	1.75 [0.07]	1.78 [0.02]	1.78 [0.02]	1.90 [0.12]	1.89 [0.15]	1.93 [0.14]	2.20 [0.33]	2.38 [0.40]
2	1 [0]	1.18 [0.34]	1.25 [0.30]	1.47 [0.41]	1.65 [0.44]	1.78 [0.59]	1.88 [0.51]	1.89 [0.52]	1.95 [0.51]	1.97 [0.53]	2.03 [0.55]	2.21 [0.71]	2.29 [0.69]
3	1 [0]	1.13 [0.09]	1.17 [0.12]	1.03 [0.11]	1.04 [0.23]	0.97 [0.28]	0.83 [0.38]	0.63 [0.46]	0.52 [0.37]	0.37 [0.21]	0.28 [0.18]	0.26 [0.19]	0.25 [0.26]
4	1 [0]	1.18 [0.11]	1.28 [0.21]	1.09 [0.18]	1.09 [0.19]	1.10 [0.32]	0.96 [0.49]	0.77 [0.45]	0.57 [0.46]	0.58 [0.48]	0.53 [0.47]	0.55 [0.54]	0.57 [0.61]
5	1 [0]	1.18 [0.17]	1.22 [0.21]	1.10 [0.18]	1.05 [0.2]	0.96 [0.17]	0.89 [0.31]	0.58 [0.17]	0.46 [0.19]	0.35 [0.18]	0.31 [0.14]	0.26 [0.12]	0.26 [0.17]

Table 5 Relative tumor Volume in an NCI-N87 advanced stage tumor model

The tumor volume at day x is expressed as Relative Tumor Volume (RTV) and calculated according to the following formula: $RTV = TV_x/TV_{22}$, where TV_x is the tumor volume at day x and TV_{15} is the tumor volume before the start of treatment on Day 22.

Group		D22	D23	D24	D25	D26	D27	D29
Gr2 Vehicle	Median	192.18	216.95	247.22	277.8	293.59	293.79	317.17
	T/C (%)	100	100	100	100	100	100	100
Gr1 Vehicle w/o T cells	Median	175.12	211.51	230.4	233.38	268.74	281.14	303.35
	T/C (%)	91	98	93	84	92	96	96
Gr3 MTR118 2.5 mg/kg	Median	194.8	211.97	229.7	200.1	195.76	209.25	159.42
	T/C (%)	101	98	93	72	67	71	50
Gr4 MTR118 0.5 mg/kg	Median	194.24	248.82	247.92	217.43	204.03	203.09	201.47
	T/C (%)	101	115	100	78	70	69	64
Gr5 MTR118 0.1 mg/kg	Median	194.64	207.02	227.09	193.2	195.69	200.65	181.06
	T/C (%)	101	95	92	70	67	68	57

Group		D30	D31	D33	D36	D38	D40	D43
Gr2 Vehicle	Median	340.66	338.43	349.83	361.18	366.69	383.84	397.1
	T/C (%)	100	100	100	100	100	100	100
Gr1 Vehicle w/o T cells	Median	308.46	317.80	316.75	322.11	358.04	374.32	421.2
	T/C (%)	91	94	91	89	98	98	106
Gr3 MTR118 2.5 mg/kg	Median	154.53	89.08	64.43	56.17	43.64	38.84	32.18
	T/C (%)	45	26	18	16	12	10	8
Gr4 MTR118 0.5 mg/kg	Median	151.36	104.15	63.6	63.59	53.01	50.11	42.05
	T/C (%)	44	31	18	18	14	13	11
Gr5 MTR118 0.1 mg/kg	Median	153.39	118.11	77.48	53.43	52.82	43.16	35.01
	T/C (%)	45	35	22	15	14	11	9

Table 6 bispecific antibody-mediated tumor growth inhibition in a NCI-N87 advanced stage tumor model

The T/C% is determined by calculating TV as $T/C\% = 100 \times (\text{median TV of treated group})/(\text{median TV of control group})$.

SEQ ID NO.	DESIGNATION	SOURCE or FORMAT	TYPE	SEQUENCE
1.	Human 5T4	human	nn	atgcctgggggtgctccccggggccccgcgcggggaacggggtctgcgggtgcgcgact agcgctggtactcctgggctgggtctcctcgtcttctccacacctcctcctcctctct cctcctcggcgcgttcctggcttcggcgttcggcgtgtccgccccgcgtgcggaccagtgc ccgcgctgtcgagtgtccgaggcagcgcgcacagtcaagtgcgttaaccgcaatctgac cgaggtgccacggacctgccgcctacgtgcgcaacctctccttacgggcaaccagctgg ccgtgctccctgcgggccttcgccccgcgcgcgcgtggcgagctggcgcgctcaaac ctcagcggcagccctggacgaggtgcgcgggggccttcgagcatctgccagcctgcg ccagctcgacctcagccacaacccactggccgacctcagtccttcgcttctcgggcagca atgccagcgtctcgccccccagtcacctgtggaactgatcctgaaccacatcgtgccccct gaagatgagcggcagaacggagcttcgagggcatggtggtggcgccctgctggcgggccg tgactgcaggggctccgcgcttgagctggcagcaaccacttcctttacctgccgcggg atgtgtggcccaactgcccagcctcaggcacctggacttaagtaataatcgtggtgagc ctgacctacgtgtccttcgcgaacctgacacatctagaaagcctccacctggaggacaatgc cctcaaggtccttcacaatggcacccctggctgagtgcaaggtctacccccacattagggtt tcctggacaacaatccctgggtctgcgactgccacatggcagacatggtgacctggtcaag gaaacagaggtagtcaggggcaaaagaccggctcacctgtgcataatcccgaaaaaatgaggaa tcgggtcctcttggaactcaacagtgctgacctggactgtgacccgattctcccccatccc tgcaaacctcttatgtcttcctgggtattgttttagccctgataggcgctatttccctcctg gtttgtatttgaaacgcgaagggtataaaaaagtggatgcataacatcagagatgcctgcag ggatcacatggaaagggtatcattacagatatgaaatcaatgcgggaccccgagattaacgaaac tcagttctaactcggatgtctga
2.	Human 5T4	human	aa	mpggcsrgpaagdgrlrarlalvlglwvsssstssassfsssapflasavsagpplpdq palceceaaartvkcnrnltevptdlpayvrnlfltgnqlavlpagafarrpplaelaaln lsgsrlddevragafehlpslrqldlshnpladlspfafsgsnasvsapsplvelilnhivpp ederqnrsfegmvvaallagralqglrrlelasnhflylprdvlaqlpslrhldlsnslvs ltyvsfrnlthleslhlednalkvlhngtlaelqglphirvflnnpwvcdchmadmvtwlk etevvqgkdrltcaypekmrnrvillelnsadldcdpilppslqtsyvfglvialigailfl vlylnrkgikkmhnrldacrhmegyhryeinadprltnlssnsdv
3.	Cynomolgus 5T4	cynomolgus	nn	atgcctgggggtgctccccggggccccgcgcgcggggaacggggtgcgcgactgcgcgact ggcgctgggttctcctgggctgggtctcctcgtcttctccacctcctcctcctcctcct cctcctcggcgcgttcctggcttcgcgcgctccgcgcgtcccgccccgcgtgcccgaccagtgt cccgcgtttgcgagtgtccgagggcgcgcacagtcaagtgcgttaaccgcaatctgac cgaggtgccacggacctgccctctacgtgcgcaacctctccttacgggcaaccagctgg

				cggtgctccctgctggcgccctcgcccgccggcccgctggcgagctggctgcgctcaac ctcagcggcagccgctggacgagtgcgcgggggcgccctcgagcatctgccagcctgcg ccagctcgacctcagccacaaccactggcctacctcagcccttcgctttctcgggcagca atgccagcatctcgccccccagtcacctgtggaactgatcctgaaccacatcgtgccccct gatgataagcggcagaaacggagcttcgagggcatggtggcgggccctggtggcgggccg tgactgcaggggctccacctcctggagctggccagcaaccacttcctttacctgcgcggg atgtgctggcccaactgccagcctcaggtacctggacttaagtaacaattcgctggtgagc ctgacctacgtgccttcgcgaacctgacacatctagaaagcctccacctggaggacaatgc cctcaagggtccttcacaaatggcacccctggctgagttgcaaggctgccccacgttagggtct tcctggacaacaatccctgggtttgcgactgccacatggcagacatggtgacctggtcaag caaacaggggtggtgcagggcaaaagaccggctcacctgtgctttccagaaaaaatgaggaa tcgggtcctcttggaactcaacagtgccgacctggactgtgacctatcttcccccatccc tgcaaacctcttatgtcttcctgggtattgttttagccctgataggcgctattttcctcctg gttttgatattgaaccgcaaggggataaaaaagtggatgcataaacatcagagatgcctgcag ggatcacatggaagggtatcattacagatatgaaatcaatgcagacccccaggttaacaaacc tcagttctaactcggatgtctga
4.	Cynomolgus 5T4	cynomolgus	aa	mpggcsrgpaagdgrlrarlarlalvllgwssssstssassssapflasaasaqplpdqc palceceaaartvkcnrnlttevptdlplyvrnlfltnqlavlpagafarrpplaelaaln lsgsrlddevrggafehlpslrqlldlshnplaylspfafsgsnasisapsplvelilnhihpp ddkrqnrsfegmvaalvagralqglhllelasnhflylprdvlaqlpslryldlsnnsivs ltyvsfrnlthleslhlednalkvlhngtlaelqglphvrvfldnnpwvcdchmadmvtwlk qtgvvqgkdrltcafpckmrnrvillelnsaddldecpilppslqtsyvfllgialigaifl vlylnrkgiikkwmhnirdacrdhmeghyryeinadprltnlssnsdv
5.	Rhesus 5T4	rhesus	nn	atgcctggggggtgctccccggggcccgcccgccgggacgggctgcggctggcgcgact ggcgctggttctcctgggctgggtctcctcgctcttctccacctcctcggcatcctcctct cctcctcgcgccgttcctggcttcggccgctccggccagcccccgctgcccgaccagtgt cccgcgcttgcgagtgctccgagggcgcgacagtcgaagtgcgttaacccgcaatctgac cgagtgcccaaggacctgccccctctacgtgcgcaacctcttccttacgggcaaccagctgg ccgtgctccctgcggcgcccttcgcccgcggcccgccgctggcgagctggctgcgctcaac ctcagcggcagccgctggacgaggtgcgcgcggcgcccttcgagcatctgccagcctgcg ccagctgacctcagccacaacccactggcctacctcagcccccttcgctttctcgggcagca atgccagcatctcggccccccagtcaccttgaggaaactgatcctgaaccacatcgtgccccct gatgataagcggcagaaacggagcttcgagggcatggtggcgggccctggtggcgggccg tgactgcaggggctccacctcctggagctggccagcaaccacttcctttacctgcgcggg atgtgctggcccaactgccagcctcaggtacctggacttaagtaacaattcgctggtgagc ctgacctacgtgtccttcgcgaacctgacacatctagaaagcctccacctggaggacaatgc cctcaagggtccttcacaatggcacccctggctgagttgcaagggtcgtccccacgttagggtct

				tcctggacaacaatccctgggtttgcgactgccacatggcagacatggtgacctggctcaag caaacagaggtggtgcagggcacaagaccggtcacctgtgcttttcccgaataatgaggaa tcgggtcctcttggaactcaacagtccgacctggactgtgacccgattcttcccccatccc tgcaaacctcttatgtcttcctgggtattgttttagccctgataggcgctattttcctcctg gttttgatatgaaccgcaaggggataaaaaagtggtgcataaacatcacagatgcctgcag ggatcacatggaagggtatcattacagatatgaaatcaatcgggaccccgaggttaacaaacc tcagttctaactcggatgtctga
6.	Rhesus 5T4	rhesus	aa	mpggcsrppaagdgrlrllarlalvllgwsssstssassfsssapflasaasaqplpdqc palceceaaartvkcvnrnlttevptdlplyvrnlfltnqlavlpagafarrpplaelaaln lsgsrldevragafehlpslrqldlshnplaylspfafsgsnasisapsplvelilnhivpp ddkrqnrsfegmvaaalvagralqglhllelasnhfllylprdvlaqlpslryldlsnnsivs ltyvsfrnlthleslhlednalkvlhngtlaelqglphvrvfldnnpwvcdchmadmvtwlk qtevvgkdrlltcafpekmrnrvillelnsaddlcdpilppslqtsyvfglgivaligaifl vlylnrkgiikkwmhnirdacrdhmegyhryeinadprltnlssndv
7.	Mouse 5T4	murine	nn	atgcctggggcggtccccggggccctccgcggggacggacggctgaggttggcaaggct ggcgctagtgtctgggttgggtctccgcgtcgccccccagctcttcggtacctcgtctt ccacctccccgcagccttcctggcctcgggtctgcgcagcctccgcagccgagagatgc ccgcggtgcgagtgctccgagggcgcgacggttaagtgcgtgaaccgcaacctgct ggaggtccggcggtatctaccgccttacgtgcgcaacctttccttacggcaaccagatga ccgtgctccccggggcgcttcgcccccgagcccgctcgccgacctggaggcgctcaac ctcagcggcaaccacctgaaggaaagtgtgtcaggtgccttcgagcatctgccgggtctgcg ccggcttgacctcagccacaaccctctcaccaacctcagcgcttcgccttgcgggcagca acgccagcgtctcggcccccgagccccctggaggagctgacccgaatcacatcgtgccccct gaggatcagaggcagaaacgggagcttcgaggggtatggtggccttcgaaggcatggtggcagc agctctgcgctcaggccttgcaactccgaggtcttacacgcctggagctagccagcaatcact ttctttcctgcctcgggacttactagcccaactgccgagctcagataacctggacctcagg aacaattccctggtgagcctgacctacgcatccttcgcgaacctgacacacctcgaaagcct ccacttggaggacaatgccctcaaggctcctcacaaacctccaccttggctgagtggcacggcc tggctcatgtcaagggtgttcctggacaacaatccctgggtttgcgactgctacatggctgac atggtggcttggcttaaagagacagaggtggtgccagataaaagccaggcttacctgcgcatt ccggagaaaaatgaggaaatcgtggcctcttagacctcaacagctctgacctggactgtgacg ctgtccttccccaatccctgcagacttcctatgtcttcctaggtattgttttagcttgata ggcgctattttcctcctcgttttgtatttgaaaccgtaaaaggcataaaaaagtggatgcataa catcacagatgcctgcagggtcacatggaagggtatcattacagatacgaaatcaatgcgg acccagatataacaaatcttagttccaactcggatgtctga
8.	Mouse 5T4	murine	aa	mpgagsrgpsagdgrlrllarlalvllgwvsasapsssvpssstspaaflasgsaqpppaerc paacecseaaartvkcvnrnlllevpadlppyvrnlfltnqmtvlpagafarqppladlealn

				lsqnhlkevcaqafehlpglrrldlshnpltnlsafagsnasvsapspleelilnhihpp edqrngsfegmvafegmvaaalrsglalrgltrlelasnhflflprdl1aqplsryldlr nns1vsltyasfrnlthleslhlednalkvlnhnstlaewhglahvkvfldnnpwvdcymad mvawlketevvdpkarltcafpekmnrnglldlnssdlcdavlpqslqtsyvflg1vlali gai1fl1vlylnrkgi1kkwmhni1rdacrdhmeghyryeinadprltnlssnsdv
9.	5T4-1 HL x CD3 HL	bispecific molecule	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYY AESVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAMDYWGQGT1VTVSS GGGSGGGSGGSDIQMTQSPSSLSASVGDRTITCKASQDINSYLSWFQKQPGKAPKIL IYRANRLVDGVPSRFSGSGSGKDFTTISSLQPEDIAITYCLQYDYLPLTFGGGTKVEIKSG GGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFGNSYISYWAYWGQGT1 VTVSSGGGSGGGSGGGSQTVVTEPESLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQKQP GQAPRGLIGGTFKFLAPGTPARFSGSLLGKAA1TL1SGVQPEDEAEYYCVLWYSNRWVFGGGT KLTVL
10.	5T4-1 HL	scFv	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYY AESVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAMDYWGQGT1VTVSS GGGSGGGSGGSDIQMTQSPSSLSASVGDRTITCKASQDINSYLSWFQKQPGKAPKIL IYRANRLVDGVPSRFSGSGSGKDFTTISSLQPEDIAITYCLQYDYLPLTFGGGTKVEIK
11.	5T4-1 H	VH	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYY AESVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAMDYWGQGT1VTVSS
12.	5T4-1 L	VL	aa	DIQMTQSPSSLSASVGDRTITCKASQDINSYLSWFQKQPGKAPKIL1YRANRLVDGVPSRF SGSGSGKDFTTISSLQPEDIAITYCLQYDYLPLTFGGGTKVEIK
13.	5T4-1 VH CDR1	VH CDR1	aa	NYWMN
14.	5T4-1 VH CDR2	VH CDR2	aa	EIRLKSDNYATYYAESVKG
15.	5T4-1 VH CDR3	VH CDR3	aa	RRGYGSAYAMDY
16.	5T4-1 VL CDR1	VL CDR1	aa	KASQDINSYLS
17.	5T4-1 VL CDR2	VL CDR2	aa	RANRLVD
18.	5T4-1 VL CDR3	VL CDR3	aa	LQYDYLPLT
19.	5T4-2 HL x CD3 HL	bispecific molecule	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDKYATYY AESVKGRFTISRDDSKNSLYLQMNNLKTEDTGVIYCARRRGYGSAYGM1DYWGQGT1VTVSS GGGSGGGSGGSDIQMTQSPSSLSASVGDRTITCKASQDINSYLSWFQKQPGKAPKIL IYRANRLVDGVPSRFSGSGSGKDFTTISSLQPEDIAITYCLQYDYLPLTFGGGTKVEIKSG GGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNY

				ATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFNGNSYISYWAYWGQGTLLVTVSSGGGGGGGGGGSGQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTFKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
20.	5T4-2 HL	scFv	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDKYATYYAESVKGRFTISRDDSKNSLYLQMNNLKTEDTGVIYCARRRGYGSAYGM DYWGQGTLLVTVSSGGGGGGGGGGGGDIQMTQSPSSLSASVGDRTITCKASQDINSYLSWVQQKPGKAPKIL IYRANRLVDGVPSRFSGSGGKDFTFITSSLPEDIAITYYCLQYDYLPLTFGGGTKVEIK
21.	5T4-2 H	VH	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDKYATYYAESVKGRFTISRDDSKNSLYLQMNNLKTEDTGVIYCARRRGYGSAYGM DYWGQGTLLVTVSS
22.	5T4-2 L	VL	aa	DIQMTQSPSSLSASVGDRTITCKASQDINSYLSWVQQKPGKAPKIL IYRANRLVDGVPSRFSGSGGKDFTFITSSLPEDIAITYYCLQYDYLPLTFGGGTKVEIK
23.	5T4-2 VH CDR1	VH CDR1	aa	NYWMN
24.	5T4-2 VH CDR2	VH CDR2	aa	EIRLKSDKYATYYAESVKG
25.	5T4-2 VH CDR3	VH CDR3	aa	RRGYGSAYGMDY
26.	5T4-2 VL CDR1	VL CDR1	aa	KASQDINSYLS
27.	5T4-2 VL CDR2	VL CDR2	aa	RANRLVD
28.	5T4-2 VL CDR3	VL CDR3	aa	LQYDYLPLT
29.	5T4-4 HL x CD3 HL	bispecific molecule	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYYAESVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAM DYWGQGTLLVTVSSGGGGGGGGGGGGDIQMTQSPSSLSASVGDRTITCKASQDINSYLSWVQQKPGKAPKIL IYRANRLVDGVPSRFSGSGGKDFTFITSSLPEDIAITYYCLQYDYLPLTFGGGTKVEIKSGGGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFNGNSYISYWAYWGQGTLLVTVSSGGGGGGGGGGGGSGQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTFKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
30.	5T4-4 HL	scFv	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYYAESVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAM DYWGQGTLLVTVSSGGGGGGGGGGGGDIQMTQSPSSLSASVGDRTITCKASQDINSYLSWVQQKPGKAPKIL IYRANRLVDGVPSRFSGSGGKDFTFITSSLPEDIAITYYCLQYDYLPLTFGGGTKVEIK
31.	5T4-4 H	VH	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYYAESVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAM DYWGQGTLLVTVSS

32.	5T4-4 L	VL	aa	DIQMTQSPSSLSASVGDRTVITCKASQDINSYLSWFQQKPGKAPKILIYRANRLVDGVPSRF SGSGSGKDFTTISSLQPEDIAITYYCLQYDFLPLTFGGGKVEIKGGSGGGGGGGSEV
33.	5T4-4 VH CDR1	VH CDR1	aa	NYWMN
34.	5T4-4 VH CDR2	VH CDR2	aa	EIRLKSDNYATYYAESVKG
35.	5T4-4 VH CDR3	VH CDR3	aa	RRGYGSAYAMDY
36.	5T4-4 VL CDR1	VL CDR1	aa	KASQDINSYLS
37.	5T4-4 VL CDR2	VL CDR2	aa	RANRLVD
38.	5T4-4 VL CDR3	VL CDR3	aa	LQYDFLPLT
39.	5T4-4 LH	scFv	aa	DIQMTQSPSSLSASVGDRTVITCKASQDINSYLSWFQQKPGKAPKILIYRANRLVDGVPSRF SGSGSGKDFTTISSLQPEDIAITYYCLQYDFLPLTFGGGKVEIKGGSGGGGGGGSEV QLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYYAE SVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAMDYWGQGLTVTVSS SVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAMDYWGQGLTVTVSS
40.	5T4-4 LH x CD3 HL	bispecific molecule	aa	DIQMTQSPSSLSASVGDRTVITCKASQDINSYLSWFQQKPGKAPKILIYRANRLVDGVPSRF SGSGSGKDFTTISSLQPEDIAITYYCLQYDFLPLTFGGGKVEIKGGSGGGGGGGSEV QLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYYAE SVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAMDYWGQGLTVTVSSSG GGSEVQLVESGGGLVQPGGSLRLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFNGNSYISYWAYWGQGL VTVSSGGGGSGGGSGGSQTVVTQEPSTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKP GQAPRGLIGGTFKFLAPGTPARFSGSLLGKAAALTLSGVQPEDEAEYVCVLWYSNRWVFGGTT KLTVL
41.	5T4-4 LH x CD3 LH	bispecific molecule	aa	DIQMTQSPSSLSASVGDRTVITCKASQDINSYLSWFQQKPGKAPKILIYRANRLVDGVPSRF SGSGSGKDFTTISSLQPEDIAITYYCLQYDFLPLTFGGGKVEIKGGSGGGGGGGSEV QLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYYAE SVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAMDYWGQGLTVTVSSSG GGSQTVVTQEPSTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGAPRGLIGGTFKFLAP GTPARFSGSLLGKAAALTLSGVQPEDEAEYVCVLWYSNRWVFGGKTLTVLGGSGGGGGSG GGSEVQLVESGGGLVQPGGSLRLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFNGNSYISYWAYWGQGL VTVSS
42.	5T4-4 HL x CD3 LH	bispecific molecule	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWVRQAPGKGLEWVGEIRLKSDNYATYY AESVKGRFTISRDDSKNSVYLQMNNLKTEDTGVIYCARRRGYGSAYAMDYWGQGLTVTVSS GGGGSGGGSGGGSDIQMTQSPSSLSASVGDRTVITCKASQDINSYLSWFQQKPGKAPKIL

				IYRANRLVDGVPSRFSGSGSGKDFTTISSLQPEDIATYYCLQYDFLPLTFGGGKVEIKSG GGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWWVQQKPGQAPRGLIGGKFLAP GTPARFSGLLGGKAALTLSGVQPEDEAEYCVLWYSNRWVFGGKTLTVLGGGSGGGSG GGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFGNSYISYWAYWGQGT LVTVSS
43.	5T4-3 HL x CD3 HL	bispecific molecule	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWWVRQAPGKGLEWVGEIRLKSDKYATYY AESVKGRFTISRDDSKNSLYLQMNNLKTEDTGVIYCARRRGYGSAYGMDYWGQGT LVTVSSGGGGGGGGSDIQMTQSPSSLSASVGDRTITCKASQDINSYLSWFQKPGKAPKIL IYRANRLVDGVPSRFSGSGSGKDFTTISSLQPEDIATYYCLQYDFLPLTFGGGKVEIKSG GGSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWWVRQAPGKGLEWVARIRSKYNNY ATYYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGFGNSYISYWAYWGQGT LVTVSSGGGGGGGGSGQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWWVQQK PQAPRGLIGGKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYCVLWYSNRWVFGG GTKLTVL
44.	5T4-3 HL	scFv	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWWVRQAPGKGLEWVGEIRLKSDKYATYY AESVKGRFTISRDDSKNSLYLQMNNLKTEDTGVIYCARRRGYGSAYGMDYWGQGT LVTVSSGGGGGGGGSDIQMTQSPSSLSASVGDRTITCKASQDINSYLSWFQKPGKAPKIL IYRANRLVDGVPSRFSGSGSGKDFTTISSLQPEDIATYYCLQYDFLPLTFGGGKVEIK
45.	5T4-3 H	VH	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTFSNYWMNWWVRQAPGKGLEWVGEIRLKSDKYATYY AESVKGRFTISRDDSKNSLYLQMNNLKTEDTGVIYCARRRGYGSAYGMDYWGQGT LVTVSS
46.	5T4-3 L	VL	aa	DIQMTQSPSSLSASVGDRTITCKASQDINSYLSWFQKPGKAPKILIRANRLVDGVPSRF SGSGSGKDFTTISSLQPEDIATYYCLQYDFLPLTFGGGKVEIK
47.	5T4-3 VH CDR1	VH CDR1	aa	NYWMN
48.	5T4-3 VH CDR2	VH CDR2	aa	EIRLKSDKYATYYAESVKG
49.	5T4-3 VH CDR3	VH CDR3	aa	RRGYGSAYGMDY
50.	5T4-3 VL CDR1	VL CDR1	aa	KASQDINSYLS
51.	5T4-3 VL CDR2	VL CDR2	aa	RANRLVD
52.	5T4-3 VL CDR3	VL CDR3	aa	LQYDFLPLT
53.	5T4-5 HL x CD3 HL	bispecific molecule	aa	EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYWMTWVRQAPGKGLEWVGEINPDSSKIN YDPSLKDKFTISRDNAKNTLYLQMNLSRAEDTAVYYCARDYGYGLAYWGQGT LVTVSSGGGSGGSGGGSDIVMTQTPLSSPVTLGQPVVISCRSSQSLANSYNTYLSWYQ QRPQGQSPRLLIYGISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGT HQPYTFGGQGT KLEIKSGGGG

					GSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGNSYISYWAYWGQGLVT VSSGGSGGGSGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQ APRGLIGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGTKL TVL
54.	5T4-5 HL	scFv	aa		EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYWMTWVRQAPGKGLEWVGEINPDSSKINYDP SLKDKFTISRDNAKNTLYLQMNSLRAEDTAVYYCARDYGYGLAYWGQGLVTVSSGGSGG GGSGGSDIVMTQTPLSPPVTLGQPVSISCRSSQSLANSYGNTYLSWYQQRPQGSPRLLIY GISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGTHTQPYTFGGQGTKLEIK
55.	5T4-5 H	VH	aa		EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYWMTWVRQAPGKGLEWVGEINPDSSKINYDP SLKDKFTISRDNAKNTLYLQMNSLRAEDTAVYYCARDYGYGLAYWGQGLVTVSS
56.	5T4-5 L	VL	aa		DIVMTQTPLSPPVTLGQPVSISCRSSQSLANSYGNTYLSWYQQRPQGSPRLLIYGISNRFSG VPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGTHTQPYTFGGQGTKLEIK
57.	5T4-5 VH CDR1	VH CDR1	aa		RYWMT
58.	5T4-5 VH CDR2	VH CDR2	aa		EINPDSSKINYDPSLKD
59.	5T4-5 VH CDR3	VH CDR3	aa		DYGYGLAY
60.	5T4-5 VL CDR1	VL CDR1	aa		RSSQSLANSYGNTYLS
61.	5T4-5 VL CDR2	VL CDR2	aa		GISNRFs
62.	5T4-5 VL CDR3	VL CDR3	aa		LQGTHQPYT
63.					EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYWMTWVRQAPGKGLEWVGEINPDSSKINYPP SLKDKFTISRDNAKNTLYLQMNSLRAEDTAVYYCARDYGYGLAYWGQGLVTVSSGGSGG GGSGGSDIVMTQSPLSPPVTLGDPASISCRSSQSLANSYGNTYLSWYQQRPQGPPRLLIY GISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGTHTQPYTFGGQGTKLEIKSGG GSEVQLVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGNSYISYWAYWGQGLVT VSSGGSGGGSGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQ APRGLIGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGTKL TVL
64.	5T4-6 HL x CD3 HL	bispecific molecule	aa		EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYWMTWVRQAPGKGLEWVGEINPDSSKINYPP SLKDKFTISRDNAKNTLYLQMNSLRAEDTAVYYCARDYGYGLAYWGQGLVTVSSGGSGG GGSGGSDIVMTQSPLSPPVTLGDPASISCRSSQSLANSYGNTYLSWYQQRPQGPPRLLIY GISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGTHTQPYTFGGQGTKLEIK
65.	5T4-6 H	VH	aa		EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYWMTWVRQAPGKGLEWVGEINPDSSKINYPP

						SLKDKFTISRDNAKNTLYLQMNSLRAEDTAVYYCARDYGYGLAYWGQGTLLVTVSS
66.	5T4-6 L		VL	aa		DIVMTQSPVLTIGDPASISCRSSQSLANSYGNTYLSWYQQRPQPRLLIYGISNRFSG VPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGTHQPYTFGGQGTKLEIK
67.	5T4-6 VH CDR1		VH CDR1	aa		RYWMT
68.	5T4-6 VH CDR2		VH CDR2	aa		EINPDSSKINYPPSLKD
69.	5T4-6 VH CDR3		VH CDR3	aa		DYGYGLAY
70.	5T4-6 VL CDR1		VL CDR1	aa		RSSQSLANSYGNTYLS
71.	5T4-6 VL CDR2		VL CDR2	aa		GISNRFS
72.	5T4-6 VL CDR3		VL CDR3	aa		LQGTHQPYT
73.						EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYMTWVRQAPGKGLEWVGEINPDSSKINYAP SLKDKFTISRDNAKNTLYLQMNSLRAEDTAVYYCARDYGYGLAYWGQGTLLVTVSSGGGSGG GGSGGGSDIVMTQSPVLTIGDPASISCRSSQSLANSYGNTYLSWYQQRPQPRLLIY GISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGTHQPYTFGGQGTKLEIKSGGG GSEVQLVESGGGLVQPGGSLRLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYAT YYADSVKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNGNSYISYWAYWGQGTLLVT VSSGGGSGGGSGGGGQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQ APRGLIGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKL TVL
74.	5T4-7 HL x CD3 HL		bispecific molecule	aa		EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYMTWVRQAPGKGLEWVGEINPDSSKINYAP SLKDKFTISRDNAKNTLYLQMNSLRAEDTAVYYCARDYGYGLAYWGQGTLLVTVSSGGGSGG GGSGGGSDIVMTQSPVLTIGDPASISCRSSQSLANSYGNTYLSWYQQRPQPRLLIY GISNRFSGVPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGTHQPYTFGGQGTKLEIK
75.	5T4-7 H		VH	aa		EVQLVESGGGLVQPGGSLRLSCAASGFTLSRYMTWVRQAPGKGLEWVGEINPDSSKINYAP SLKDKFTISRDNAKNTLYLQMNSLRAEDTAVYYCARDYGYGLAYWGQGTLLVTVSS
76.	5T4-7 L		VL	aa		DIVMTQSPVLTIGDPASISCRSSQSLANSYGNTYLSWYQQRPQPRLLIYGISNRFSG VPDRFSGSGAGTDFTLKISRVEAEDVGVYYCLQGTHQPYTFGGQGTKLEIK
77.	5T4-7 VH CDR1		VH CDR1	aa		RYWMT
78.	5T4-7 VH CDR2		VH CDR2	aa		EINPDSSKINYAPSLKD
79.	5T4-7 VH CDR3		VH CDR3	aa		DYGYGLAY
80.	5T4-7 VL CDR1		VL CDR1	aa		RSSQSLANSYGNTYLS

81.	5T4-7 VL CDR2	VL CDR2	aa	GISNRFS	
82.	5T4-7 VL CDR3	VL CDR3	aa	LQGTHQPYT	
83.				QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWVRQPPGKGLEWIGVIWASGRTNYNP LMSRVTISGDTSKNQVSLKLSVTAADTAVYYCAGSNWGYAMDYWGQGLVTVSSGGGSGG GGSGGGSAIRMTQSPSSFASAGDRVTITCRASENI SYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYSTPYTFGQGTKLEIKSGGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLVTVSSGG GGSGGSGGGGQTVVTQEPSLTVSPGGTVTLTCGSSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYCVLWYSNRWVFGGGTKLTVL	
84.	5T4-8 HL x CD3 HL	bispecific molecule	aa	QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWVRQPPGKGLEWIGVIWASGRTNYNP LMSRVTISGDTSKNQVSLKLSVTAADTAVYYCAGSNWGYAMDYWGQGLVTVSSGGGSGG GGSGGGSAIRMTQSPSSFASAGDRVTITCRASENI SYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYSTPYTFGQGTKLEIK	
85.	5T4-8 HL	scFv	aa	QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWVRQPPGKGLEWIGVIWASGRTNYNP LMSRVTISGDTSKNQVSLKLSVTAADTAVYYCAGSNWGYAMDYWGQGLVTVSS	
86.	5T4-8 H	VH	aa	AIRMTQSPSSFASAGDRVTITCRASENI SYLAWYQQKPKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYSTPYTFGQGTKLEIK	
87.	5T4-8 L	VL	aa	SYGVH	
88.	5T4-8 VH CDR1	VH CDR1	aa	VIWASGRTNYSALMS	
89.	5T4-8 VH CDR2	VH CDR2	aa	SNWGYAMDY	
90.	5T4-8 VH CDR3	VH CDR3	aa	RASENI SYLA	
91.	5T4-8 VL CDR1	VL CDR1	aa	NAKTLAE	
92.	5T4-8 VL CDR2	VL CDR2	aa	QHHYSTPYT	
93.	5T4-8 VL CDR3	VL CDR3	aa	QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGLHWIRQPPGKSLEWLGIWASGRTNYNP LMSRVTISGDTSKNQVSLKLSVTAADTAVYYCAGSNWGYAMDYWGQGLVTVSSGGGSGG GGSGGGSAIRMTQSPSSFASAGDRVTITCRASENI SYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYSTPYTFGQGTKLEIKSGGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLVTVSSGG GGSGGSGGGGQTVVTQEPSLTVSPGGTVTLTCGSSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYCVLWYSNRWVFGGGTKLTVL	
	5T4-9 HL x CD3 HL	bispecific molecule	aa		

94.	5T4-9 HL		scFv	aa	QVQLQESGPGLVKPSSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGVIWASGRTNYNPA LKSrvTISGDTskNQVSLKLSLTAADTAVYcAGSNWGYAMDYWGQGLVTVSSGGSGG GGSGGSAIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKGAPQLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYSTPYTFGGGTKLEIK
95.	5T4-9 H		VH	aa	QVQLQESGPGLVKPSSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGVIWASGRTNYNPA LKSrvTISGDTskNQVSLKLSLTAADTAVYcAGSNWGYAMDYWGQGLVTVSS
96.	5T4-9 L		VL	aa	AIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKGAPQLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYSTPYTFGGGTKLEIK
97.	5T4-9 VH CDR1		VH CDR1	aa	SYGLH
98.	5T4-9 VH CDR2		VH CDR2	aa	VIWASGRTNYNPALKS
99.	5T4-9 VH CDR3		VH CDR3	aa	SNWGYAMDY
100.	5T4-9 VL CDR1		VL CDR1	aa	RASENIYSYLA
101.	5T4-9 VL CDR2		VL CDR2	aa	NAKTLAE
102.	5T4-9 VL CDR3		VL CDR3	aa	QHHYSTPYT
103.					QVQLQESGPGLVKPSSETLSITCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWAGGRTNYN SA LMSrvTISKDTskNQVSLKLSLTAADTAVYcAGSNWGYAMDYWGQGLVTVSSGGSGG GGSGGSAIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKGAPQLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYATPLTFGGGKVEIKSGGGSEVQ LVESSGGLVQPGGSLKLSCAASGTFFNKYAMNWRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNLLKTEDTAVYCVRHGNFGNSYISYWAYWGQGLVTVSSGG GGSGGGSGGGSQTVVTEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTFKLAPGTPARFSGSLLGKAAALTLSGVQPEDEAEYCVLWYSNRWVFGGKLTVL
	5T4-10 HL x CD3 HL		bispecific molecule	aa	QVQLQESGPGLVKPSSETLSITCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWAGGRTNYN SA LMSrvTISKDTskNQVSLKLSLTAADTAVYcAGSNWGYAMDYWGQGLVTVSSGGSGG GGSGGSAIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKGAPQLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYATPLTFGGGKVEIK
104.					QVQLQESGPGLVKPSSETLSITCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWAGGRTNYN SA LMSrvTISKDTskNQVSLKLSLTAADTAVYcAGSNWGYAMDYWGQGLVTVSSGGSGG GGSGGSAIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKGAPQLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYATPLTFGGGKVEIK
105.	5T4-10 H		VH	aa	QVQLQESGPGLVKPSSETLSITCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWAGGRTNYN SA LMSrvTISKDTskNQVSLKLSLTAADTAVYcAGSNWGYAMDYWGQGLVTVSS
106.	5T4-10 L		VL	aa	AIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKGAPQLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYATPLTFGGGKVEIK
107.	5T4-10 VH CDR1		VH CDR1	aa	SYGVH
108.	5T4-10 VH CDR2		VH CDR2	aa	VIWAGGRTNYSALMS

109.	5T4-10 VH CDR3	VH CDR3	aa	SNWGYAMDY	
110.	5T4-10 VL CDR1	VL CDR1	aa	RASENIYSYLA	
111.	5T4-10 VL CDR2	VL CDR2	aa	NAKTLAE	
112.	5T4-10 VL CDR3	VL CDR3	aa	QHYYATPLT	
113.				QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWRQPPGKGLEWIGVIWASGRTNYNLSA LMSRVTISGDTSKNQVSLKLNSTAAATAVYVCAGSNWGYAMDYWGQGLVTVSSGGGSGG GGGGGSAIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGTDFTLTISSLQPEDFATYVCQHHYAIPTYFGQGTKLEIKSGGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYVCVRHGNFGNSYISYWAYWGQGLVTVSSGG GGGGGSGGSGTQVVTQEPSTLTVSPGGTDTLTCGSSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLGGKAALTLSGVQPEDEAEYCVLWYSNRWVFGGGTKLTVL	
114.	5T4-11 HL x CD3 HL	bispecific molecule	aa	QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWRQPPGKGLEWIGVIWASGRTNYNLSA LMSRVTISGDTSKNQVSLKLNSTAAATAVYVCAGSNWGYAMDYWGQGLVTVSSGGGSGG GGGGGSAIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGTDFTLTISSLQPEDFATYVCQHHYAIPTYFGQGTKLEIK	
115.	5T4-11 H	VH	aa	QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWRQPPGKGLEWIGVIWASGRTNYNLSA LMSRVTISGDTSKNQVSLKLNSTAAATAVYVCAGSNWGYAMDYWGQGLVTVSS	
116.	5T4-11 L	VL	aa	AIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTLAEGVPSRF SGSGSGTDFTLTISSLQPEDFATYVCQHHYAIPTYFGQGTKLEIK	
117.	5T4-11 VH CDR1	VH CDR1	aa	SYGVH	
118.	5T4-11 VH CDR2	VH CDR2	aa	VIWASGRTNYSALMS	
119.	5T4-11 VH CDR3	VH CDR3	aa	SNWGYAMDY	
120.	5T4-11 VL CDR1	VL CDR1	aa	RASENIYSYLA	
121.	5T4-11 VL CDR2	VL CDR2	aa	NAKTLAE	
122.	5T4-11 VL CDR3	VL CDR3	aa	QHYYAIPYT	
123.	5T4-12 HL x CD3 HL	bispecific molecule	aa	QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGLHWIRQPPGKSLEWLGIWASGRTNYNPA LKSRVTISGDTSKNQVSLKLSGVTAAATAVYVCAGSNWGYAMDYWGQGLVTVSSGGGSGG GGGGGSAIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGTDFTLTISSLQPEDFATYVCQHHYAIPTYFGQGTKLEIKSGGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWRQAPGKGLEWVARIRSKYNNYATYYADS	

				VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAWGQGTLVTVSSGG GGSGGGSGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYCVLWYSNRWVFGGKLTVL				VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAWGQGTLVTVSSGG GGSGGGSGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYCVLWYSNRWVFGGKLTVL
124.		5T4-12 HL	scFv	aa				QVQLQESGPGLVKPSSETLSITCTVSGVSISSYGLHWIRQPPGKSLLEWLGIWASGRTNYNPA LKSRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQTLVTVSSGGSGG GGSGGGSAIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTTFGQGTKLEIK
125.		5T4-12 H	VH	aa				QVQLQESGPGLVKPSSETLSITCTVSGVSISSYGLHWIRQPPGKSLLEWLGIWASGRTNYNPA LKSRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQTLVTVSS
126.		5T4-12 L	VL	aa				AIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTTFGQGTKLEIK
127.		5T4-12 VH CDR1	VH CDR1	aa				SYGLH
128.		5T4-12 VH CDR2	VH CDR2	aa				VIWASGRTNYNPALKS
129.		5T4-12 VH CDR3	VH CDR3	aa				SNWGYAMDY
130.		5T4-12 VL CDR1	VL CDR1	aa				RASENIYSYLA
131.		5T4-12 VL CDR2	VL CDR2	aa				NAKTLAE
132.		5T4-12 VL CDR3	VL CDR3	aa				QHHYAIPT
133.		5T4-12 LH	scFv	aa				AIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTTFGQGTKLEIKGGSGGGSGGGSQV QLQESGPGLVKPSSETLSITCTVSGVSISSYGLHWIRQPPGKSLLEWLGIWASGRTNYNPALK SRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQTLVTVSS
134.		5T4-12 LH x CD3 HL	bispecific molecule	aa				AIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTTFGQGTKLEIKGGSGGGSGGGSQV QLQESGPGLVKPSSETLSITCTVSGVSISSYGLHWIRQPPGKSLLEWLGIWASGRTNYNPALK SRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQTLVTVSSGGSEVQ LVESGGGLVQPGGSLKLSCAAAGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAWGQGTLVTVSSGG GGSGGGSGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYCVLWYSNRWVFGGKLTVL
135.		5T4-12 LH x CD3 LH	bispecific molecule	aa				AIRMTQSPSSFASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTTFGQGTKLEIKGGSGGGSGGGSQV QLQESGPGLVKPSSETLSITCTVSGVSISSYGLHWIRQPPGKSLLEWLGIWASGRTNYNPALK SRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQTLVTVSSGGGSQTV

				VTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFS GSLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGSGGGSGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLTVTVSS
136.	5T4-12 HL x CD3 LH	bispecific molecule	aa	QVQLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGVIWASGRTNYNPA LKSRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQGLTVTVSSGGGGSGG GGSGGGSAIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISLQPEDFATYYCQHHYAIPTTFGQGTKLEIKSGGGGSQTV VTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFS GSLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGGGSGGGSGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLTVTVSS
137.				QVQLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGVIWASGRTNYNPA LKSRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQGLTVTVSSGGGGSGG GGSGGGSAIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISLQPEDFATYYCQHHFVTPYTFGQGTKLEIKSGGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYYCVRHGNFGNSYISYWAYWGQGLTVTVSSGG GGSGGGSGGGSQTVVTQEPSLTVSPGGTVTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLGGKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
138.	5T4-13 HL x CD3 HL	bispecific molecule	aa	QVQLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGVIWASGRTNYNPA LKSRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQGLTVTVSSGGGGSGG GGSGGGSAIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISLQPEDFATYYCQHHFVTPYTFGQGTKLEIK
139.	5T4-13 H	VH	aa	QVQLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGVIWASGRTNYNPA LKSRVTISGDTSKNQVSLKLSGVTAAATAVYYCAGSNWGYAMDYWGQGLTVTVSSGGGGSGG GGSGGGSAIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISLQPEDFATYYCQHHFVTPYTFGQGTKLEIK
140.	5T4-13 L	VL	aa	AIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISLQPEDFATYYCQHHFVTPYTFGQGTKLEIK
141.	5T4-13 VH CDR1	VH CDR1	aa	SYGLH
142.	5T4-13 VH CDR2	VH CDR2	aa	VIWASGRTNYPALKS
143.	5T4-13 VH CDR3	VH CDR3	aa	SNWGYAMDY
144.	5T4-13 VL CDR1	VL CDR1	aa	RASENIYSYLA
145.	5T4-13 VL CDR2	VL CDR2	aa	NAKTLAE

146.	5T4-13 VL CDR3	VL CDR3	aa	QHHEFVTPYT AIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYQCQHHFVTPYTFGQGTKLEIKGGSGGGSGGSQV QLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGIWASGRTNYPALK SRVTISGDTSKNQVSLKLSGVTAAATAVYACAGSNWGYAMDYWGQGLVTVSS
147.	5T4-13 LH	scFv	aa	AIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYQCQHHFVTPYTFGQGTKLEIKGGSGGGSGGSQV QLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGIWASGRTNYPALK SRVTISGDTSKNQVSLKLSGVTAAATAVYACAGSNWGYAMDYWGQGLVTVSSGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYCVRHGNFGNSYISYWAYWGQGLVTVSSGG GGSGGGSGGGSQTVVTQEPSLTVSPGGTVTTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLGGKAAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
148.	5T4-13 LH x CD3 HL	bispecific molecule	aa	AIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTLAEGVPSRF SGSGGTDFTLTISSLQPEDFATYQCQHHFVTPYTFGQGTKLEIKGGSGGGSGGSQV QLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGIWASGRTNYPALK SRVTISGDTSKNQVSLKLSGVTAAATAVYACAGSNWGYAMDYWGQGLVTVSSGGGGSQTV VTQEPSLTVSPGGTVTTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFS GSLGGKAAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGSGGGSGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYCVRHGNFGNSYISYWAYWGQGLVTVSS
149.	5T4-13 LH x CD3 LH	bispecific molecule	aa	QVQLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKSLEWLGIWASGRTNYNPA LKSRVTISGDTSKNQVSLKLSGVTAAATAVYACAGSNWGYAMDYWGQGLVTVSSGGSGG GGSGGGSAIRMTQSPSSFSASAGDRVTITCRASENIYSYLAWYQQKPGKSPKLLIYNAKTL AEGVPSRFSGSGGTDFTLTISSLQPEDFATYQCQHHFVTPYTFGQGTKLEIKSGGGGSQTV VTQEPSLTVSPGGTVTTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGLIGGTKFLAPGTPARFS GSLGGKAAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVLGGSGGGSGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYCVRHGNFGNSYISYWAYWGQGLVTVSS
150.	5T4-13 LH x CD3 LH	bispecific molecule	aa	QVQLQESGGLVKPSETLSITCTVSGVSISSYGLHWIRQPPGKGLEWIGVIWSSGRTNYNPS LKSRVTISRDTSKNQVSLKLSVKAADTAVYACAGSNWGYAMDYWGQGLVTVSSGGSGG GGSGGGSAIRMTQSPSSFSASAGDRVTITCRASEDISSYLAWYQQKPGKAPKLLIYAASTL QSGVPSRFSGSGGTDFTLTISSLQPEDFATYQCQHHYAIPTYFGGQGTKLEIKSGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYCVRHGNFGNSYISYWAYWGQGLVTVSSGG GGSGGGSGGGSQTVVTQEPSLTVSPGGTVTTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL
151.	5T4-15 HL x CD3 HL	bispecific molecule	aa	QVQLQESGGLVKPSETLSITCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWSSGRTNYNPS LKSRVTISRDTSKNQVSLKLSVKAADTAVYACAGSNWGYAMDYWGQGLVTVSSGGSGG GGSGGGSAIRMTQSPSSFSASAGDRVTITCRASEDISSYLAWYQQKPGKAPKLLIYAASTL QSGVPSRFSGSGGTDFTLTISSLQPEDFATYQCQHHYAIPTYFGGQGTKLEIKSGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYCVRHGNFGNSYISYWAYWGQGLVTVSSGG GGSGGGSGGGSQTVVTQEPSLTVSPGGTVTTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL

					IGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
152.					QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWSSGRTNYNPS LKSRVTISRDTSKNQVSLKLSVKAADTAVYVCAGSNWGYAMDYWGQGLVTVVSSGGGSGG GGGGGSAIRMTQSPSSFASAGDRVTITCRASEDISSYLAWYQQKPKAPKLLIYAASTL QSGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTYFGGQGTKLEIK
153.	5T4-15 HL	scFv	aa		QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWSSGRTNYNPS LKSRVTISRDTSKNQVSLKLSVKAADTAVYVCAGSNWGYAMDYWGQGLVTVVSS
154.	5T4-15 H	VH	aa		AIRMTQSPSSFASAGDRVTITCRASEDISSYLAWYQQKPKAPKLLIYAASTLQSGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTYFGGQGTKLEIK
155.	5T4-15 L	VL	aa		
156.	5T4-15 VH CDR1	VH CDR1	aa		SYGVH
157.	5T4-15 VH CDR2	VH CDR2	aa		VIWSSGRTNYNPSLKS
158.	5T4-15 VH CDR3	VH CDR3	aa		SNWGYAMDY
159.	5T4-15 VL CDR1	VL CDR1	aa		RASEDISSYLA
160.	5T4-15 VL CDR2	VL CDR2	aa		AASTLQS
161.	5T4-15 VL CDR3	VL CDR3	aa		QHHYAIPTY
					QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWASGRTNYNPS LKSRVTISRDTSKNQVSLKLSVTAADTAVYVCAGSNWGYAMDYWGQGLVTVVSSGGGSGG GGGGGSAIRMTQSPSSFASAGDRVTITCRASEDISSYLAWYQQKPKAPKLLIYAASTL QSGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTYFGGQGTKLEIKSGGGGSEVQ LVESGGGLVQPGGSLKLSCAASGFTFNKYAMNWVRQAPGKGLEWVARIRSKYNNYATYYADS VKDRFTISRDDSKNTAYLQMNNLKTEDTAVYVCVRHGNFGNSYISYWAYWGQGLVTVSSGG GGSGGGSGGSGQT VVTQEPSLTVSPGGT VTLTCGSSTGAVTSGNYPNWVQQKPGQAPRGL IGGTKFLAPGTPARFSGSLLGKKAALTLSGVQPEDEAEYYCVLWYSNRWVFGGGTKLTVL
162.	5T4-14 HL x CD3 HL	bispecific molecule	aa		QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWASGRTNYNPS LKSRVTISRDTSKNQVSLKLSVTAADTAVYVCAGSNWGYAMDYWGQGLVTVVSSGGGSGG GGGGGSAIRMTQSPSSFASAGDRVTITCRASEDISSYLAWYQQKPKAPKLLIYAASTL QSGVPSRFSGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTYFGGQGTKLEIK
163.	5T4-14 HL	scFv	aa		QVQLQESGPGLVKPSSETLSLTCTVSGVSISSYGVHWIRQPPGKGLEWIGVIWASGRTNYNPS LKSRVTISRDTSKNQVSLKLSVTAADTAVYVCAGSNWGYAMDYWGQGLVTVVSS
164.	5T4-14 H	VH	aa		AIRMTQSPSSFASAGDRVTITCRASEDISSYLAWYQQKPKAPKLLIYAASTLQSGVPSRF SGSGGTDFTLTISSLQPEDFATYYCQHHYAIPTYFGGQGTKLEIK
165.	5T4-14 L	VL	aa		
	5T4-14 VH CDR1	VH CDR1	aa		SYGVH

166.	5T4-14 VH CDR2	VH CDR2	aa	VIWASGRITNYNPSSLKS	
167.	5T4-14 VH CDR3	VH CDR3	aa	SNWGYAMDY	
168.	5T4-14 VL CDR1	VL CDR1	aa	RASEDISSYLA	
169.	5T4-14 VL CDR2	VL CDR2	aa	AASTLQS	
170.	5T4-14 VL CDR3	VL CDR3	aa	QHHYAIPLYT	

Claims

1. A bispecific binding molecule comprising a first and a second binding domain,
 - (a) wherein the first binding domain is capable of binding to epitope cluster 4 of 5T4;
and
 - (b) wherein the second binding domain is capable of binding to the CD3 receptor complex on T cells;wherein the epitope cluster 4 corresponds to extracellular protein domain of 5T4 formed by amino acid residues 170 to 222 of the human sequence as depicted in SEQ ID NO: 2.
2. The bispecific binding molecule according to claim 1, wherein the first binding domain is capable of binding to epitope cluster 2 and epitope cluster 4 of 5T4 and wherein the epitope cluster 2 corresponds to extracellular protein domain of 5T4 formed by amino acid residues 78 to 138 of the human sequence as depicted in SEQ ID NO: 2.
3. The bispecific binding molecule according to claims 1 and 2, wherein the first binding domain is capable of binding to human and *Callithrix jacchus*, *Saguinus oedipus* or *Saimiri sciureus* 5T4 and/or the second binding domain is capable of binding to human and *Callithrix jacchus*, *Saguinus Oedipus* or *Saimiri sciureus* CD3 epsilon.
4. The bispecific binding molecule according to any one of claims 1 to 3, wherein the affinity of the first binding domain for human 5T4 is ≤ 15 nM (preferably ≤ 10 nM).
5. The bispecific binding molecule according to any one of claims 1 to 4, wherein the first and the second binding domain are derived from an antibody.
6. The bispecific binding molecule according to claim 6, which is selected from the group of (scFv)₂, (single domain mAb)₂, scFv-single domain mAb, diabody or oligomeres thereof.

7. The bispecific binding molecule according to any one of claims 1 to 6, wherein the first binding domain comprises comprises a VL region comprising CDR-L1, CDR-L2 and CDR-L3 and a VH region comprising CDR-H1, CDR-H2 and CDR-H3 selected from:
- (a) CDR-L1 as depicted in SEQ ID NO. **16**, CDR-L2 as depicted in SEQ ID NO. **17**, CDR-L3 as depicted in SEQ ID NO. **18**, CDR-H1 as depicted in SEQ ID NO. **13**, CDR-H2 as depicted in SEQ ID NO. **14** and CDR-H3 as depicted in SEQ ID NO. **15**;
 - (b) CDR-L1 as depicted in SEQ ID NO. **26**, CDR-L2 as depicted in SEQ ID NO. **17**, CDR-L3 as depicted in SEQ ID NO. **28**, CDR-H1 as depicted in SEQ ID NO. **23**, CDR-H2 as depicted in SEQ ID NO. **24** and CDR-H3 as depicted in SEQ ID NO. **25**;
 - (c) CDR-L1 as depicted in SEQ ID NO. **36**, CDR-L2 as depicted in SEQ ID NO. **37**, CDR-L3 as depicted in SEQ ID NO. **38**, CDR-H1 as depicted in SEQ ID NO. **33**, CDR-H2 as depicted in SEQ ID NO. **34** and CDR-H3 as depicted in SEQ ID NO. **35**;
 - (d) CDR-L1 as depicted in SEQ ID NO. **50**, CDR-L2 as depicted in SEQ ID NO. **51**, CDR-L3 as depicted in SEQ ID NO. **52**, CDR-H1 as depicted in SEQ ID NO. **47**, CDR-H2 as depicted in SEQ ID NO. **48** and CDR-H3 as depicted in SEQ ID NO. **49**;
 - (e) CDR-L1 as depicted in SEQ ID NO. **90**, CDR-L2 as depicted in SEQ ID NO. **91**, CDR-L3 as depicted in SEQ ID NO. **92**, CDR-H1 as depicted in SEQ ID NO. **87**, CDR-H2 as depicted in SEQ ID NO. **88** and CDR-H3 as depicted in SEQ ID NO. **89**;
 - (f) CDR-L1 as depicted in SEQ ID NO. **100**, CDR-L2 as depicted in SEQ ID NO. **101**, CDR-L3 as depicted in SEQ ID NO. **102**, CDR-H1 as depicted in SEQ ID NO. **97**, CDR-H2 as depicted in SEQ ID NO. **98** and CDR-H3 as depicted in SEQ ID NO. **99**;
 - (g) CDR-L1 as depicted in SEQ ID NO. **110**, CDR-L2 as depicted in SEQ ID NO. **111**, CDR-L3 as depicted in SEQ ID NO. **112**, CDR-H1 as depicted in SEQ ID NO. **107**, CDR-H2 as depicted in SEQ ID NO. **108** and CDR-H3 as depicted in SEQ ID NO. **109**;
 - (h) CDR-L1 as depicted in SEQ ID NO. **120**, CDR-L2 as depicted in SEQ ID NO. **121**, CDR-L3 as depicted in SEQ ID NO. **122**, CDR-H1 as depicted in SEQ ID NO. **117**, CDR-H2 as depicted in SEQ ID NO. **118** and CDR-H3 as depicted in SEQ ID NO. **119**;

- (i) CDR-L1 as depicted in SEQ ID NO. **130**, CDR-L2 as depicted in SEQ ID NO. **131**, CDR-L3 as depicted in SEQ ID NO. **132**, CDR-H1 as depicted in SEQ ID NO. **127**, CDR-H2 as depicted in SEQ ID NO. **128** and CDR-H3 as depicted in SEQ ID NO. **129**;
 - (j) CDR-L1 as depicted in SEQ ID NO. **144**, CDR-L2 as depicted in SEQ ID NO. **145**, CDR-L3 as depicted in SEQ ID NO. **146**, CDR-H1 as depicted in SEQ ID NO. **141**, CDR-H2 as depicted in SEQ ID NO. **142** and CDR-H3 as depicted in SEQ ID NO. **143**;
 - (k) CDR-L1 as depicted in SEQ ID NO. **158**, CDR-L2 as depicted in SEQ ID NO. **159**, CDR-L3 as depicted in SEQ ID NO. **160**, CDR-H1 as depicted in SEQ ID NO. **155**, CDR-H2 as depicted in SEQ ID NO. **156** and CDR-H3 as depicted in SEQ ID NO. **157**; and
 - (l) CDR-L1 as depicted in SEQ ID NO. **168**, CDR-L2 as depicted in SEQ ID NO. **169**, CDR-L3 as depicted in SEQ ID NO. **170**, CDR-H1 as depicted in SEQ ID NO. **165**, CDR-H2 as depicted in SEQ ID NO. **166** and CDR-H3 as depicted in SEQ ID NO. **167**.
8. The bispecific binding molecule according to any one of claims 1 to 7, wherein the first binding domain comprises a VL region selected from the group consisting of a VL region as depicted in SEQ ID NO: **12**, SEQ ID NO: **22**, SEQ ID NO: **32**, SEQ ID NO: **46**, SEQ ID NO: **86**, SEQ ID NO: **96**, SEQ ID NO: **106**, SEQ ID NO: **116**, SEQ ID NO: **126**, SEQ ID NO: **140**, SEQ ID NO: **154**, and SEQ ID NO: **164**.
9. The bispecific binding molecule according to any one of claims 1 to 8, wherein the first binding domain comprises a VH region selected from the group consisting of a VH region as depicted in SEQ ID NO: **11**, SEQ ID NO: **21**, SEQ ID NO: **31**, SEQ ID NO: **45**, SEQ ID NO: **85**, SEQ ID NO: **95**, SEQ ID NO: **105**, SEQ ID NO: **115**, SEQ ID NO: **125**, SEQ ID NO: **139**, SEQ ID NO: **153**, and SEQ ID NO: **163**.
10. The bispecific binding molecule according to any one of claims 1 to 9, wherein the first binding comprises a VL region and a VH region selected from the group consisting of:
- (a) a VL region as depicted in SEQ ID NO: **12** and a VH region as depicted in SEQ ID NO: **11**;

- (b) a VL region as depicted in SEQ ID NO: **22** and a VH region as depicted in SEQ ID NO: **21**;
 - (c) a VL region as depicted in SEQ ID NO: **32** and a VH region as depicted in SEQ ID NO: **31**;
 - (d) a VL region as depicted in SEQ ID NO: **46** and a VH region as depicted in SEQ ID NO: **45**;
 - (e) a VL region as depicted in SEQ ID NO: **86** and a VH region as depicted in SEQ ID NO: **85**;
 - (f) a VL region as depicted in SEQ ID NO: **96** and a VH region as depicted in SEQ ID NO: **95**;
 - (g) a VL region as depicted in SEQ ID NO: **106** and a VH region as depicted in SEQ ID NO: **105**;
 - (h) a VL region as depicted in SEQ ID NO: **116** and a VH region as depicted in SEQ ID NO: **115**;
 - (i) a VL region as depicted in SEQ ID NO: **126** and a VH region as depicted in SEQ ID NO: **125**;
 - (j) a VL region as depicted in SEQ ID NO: **140** and a VH region as depicted in SEQ ID NO: **139**;
 - (k) a VL region as depicted in SEQ ID NO: **154** and a VH region as depicted in SEQ ID NO: **153**; and
 - (l) a VL region as depicted in SEQ ID NO: **164** and a VH region as depicted in SEQ ID NO: **163**.
11. The bispecific binding molecule according to claim 10, wherein the first binding domain comprises an amino acid sequence selected from the group consisting of SEQ ID NO: **10**, SEQ ID NO: **20**, SEQ ID NO: **30**, SEQ ID NO: **39**, SEQ ID NO: **44**, SEQ ID NO: **84**, SEQ ID NO: **94**, SEQ ID NO: **104**, SEQ ID NO: **114**, SEQ ID NO: **124**, SEQ ID NO: **133**, SEQ ID NO: **138**, SEQ ID NO: **147**, SEQ ID NO: **152**, and SEQ ID NO: **162**.
12. A nucleic acid sequence encoding a bispecific binding molecule as defined in any of claims 1 to 11.
13. A vector, which comprises a nucleic acid sequence as defined in claim 12.

14. A host cell transformed or transfected with the nucleic acid sequence of claim 12 or a vector defined in claim 13.
15. A process for the production of a bispecific binding molecule according to any of claims 1 to 11, said process comprising culturing a host cell defined in claim 14 under conditions allowing the expression of the bispecific binding molecule as defined in any of claims 1 to 11 and recovering the produced bispecific binding molecule from the culture.
16. A pharmaceutical composition comprising a bispecific binding molecule according to any one of claims 1 to 11, or produced according to the process of claim 15.
17. The bispecific binding molecule according to any one of claims 1 to 11, or produced according to the process of claim 15 for use in the prevention, treatment or amelioration of a disease selected from a proliferative disease, a tumorous disease, or an immunological disorder.
18. A method for the treatment or amelioration of a disease selected from a proliferative disease, a tumorous disease, or an immunological disorder comprising the step of administering to a subject in the need thereof the bispecific binding molecule according to any one of claims 1 to 11, or produced according to the process of claim 15.
19. A kit comprising a bispecific binding molecule as defined in any of claims 1 to 11, a nucleic acid molecule as defined in claim 12, a vector as defined in any one of claims 13, or a host cell as defined in claim 14.
20. Use of amino acid residues 170 to 222 (epitope cluster 4) and/or 78 to 138 (epitope cluster 2) of the human 5T4 sequence as depicted in SEQ ID NO: 2 for the generation of an antibody.

21. A method for the generation of an antibody, preferably bispecific binding molecule, comprising
 - (a) immunizing an animal with a protein comprising amino acid residues 170 to 222 (epitope cluster 4) and/or 78 to 138 (epitope cluster 2) of the human 5T4 sequence as depicted in SEQ ID NO: 2,
 - (b) obtaining said antibody, and
 - (c) optionally converting said antibody into a bispecific binding molecule having preferably the capacity to bind to human 5T4 and CD3 as described herein.
22. A fragment of human 5T4 consisting of amino acid residues 170 to 222 (epitope cluster 4) or amino acids 78 to 138 (epitope cluster 2) of the human 5T4 sequence as depicted in SEQ ID NO: 2.

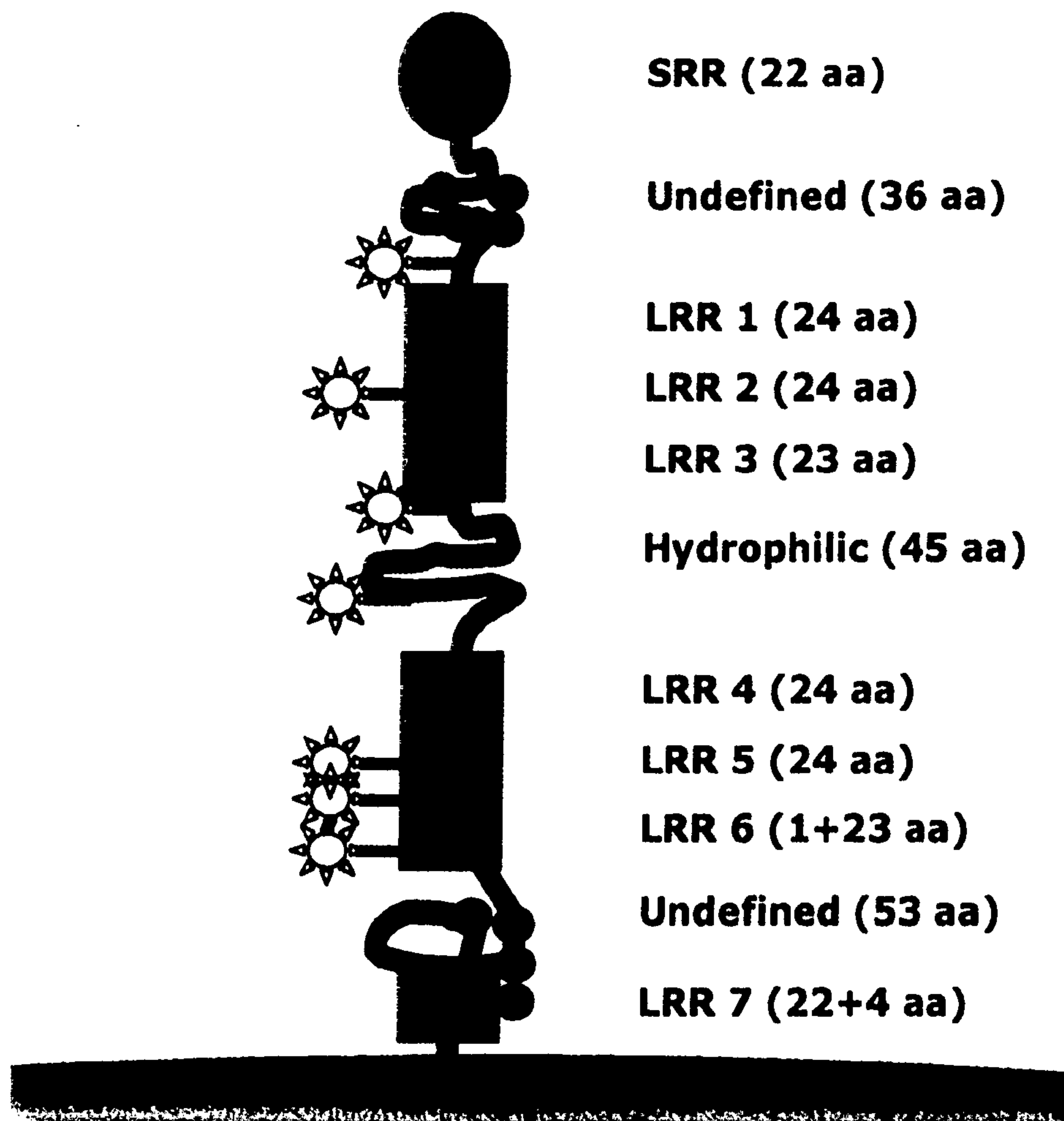
Figure 1

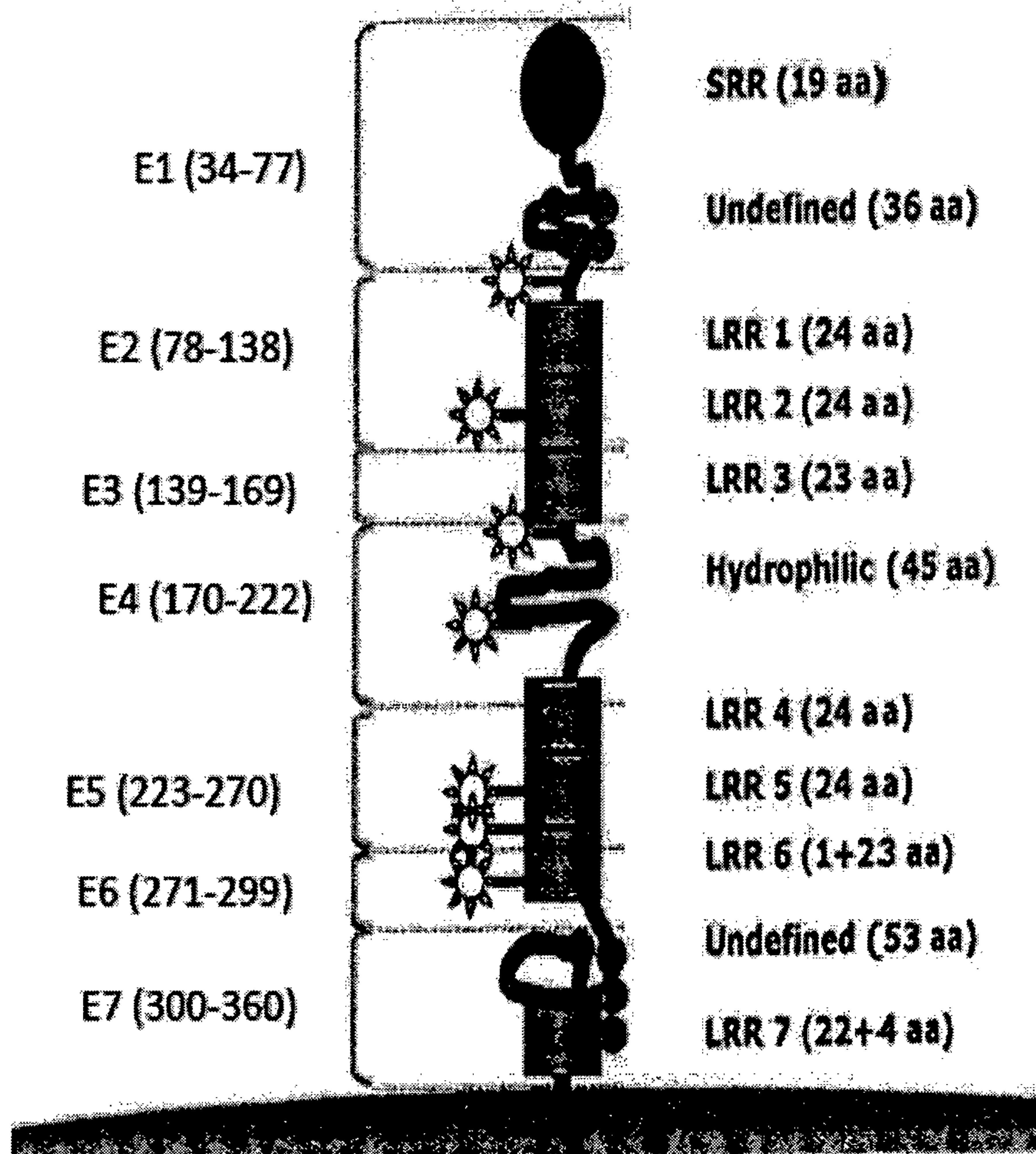
Figure 2

Figure 3

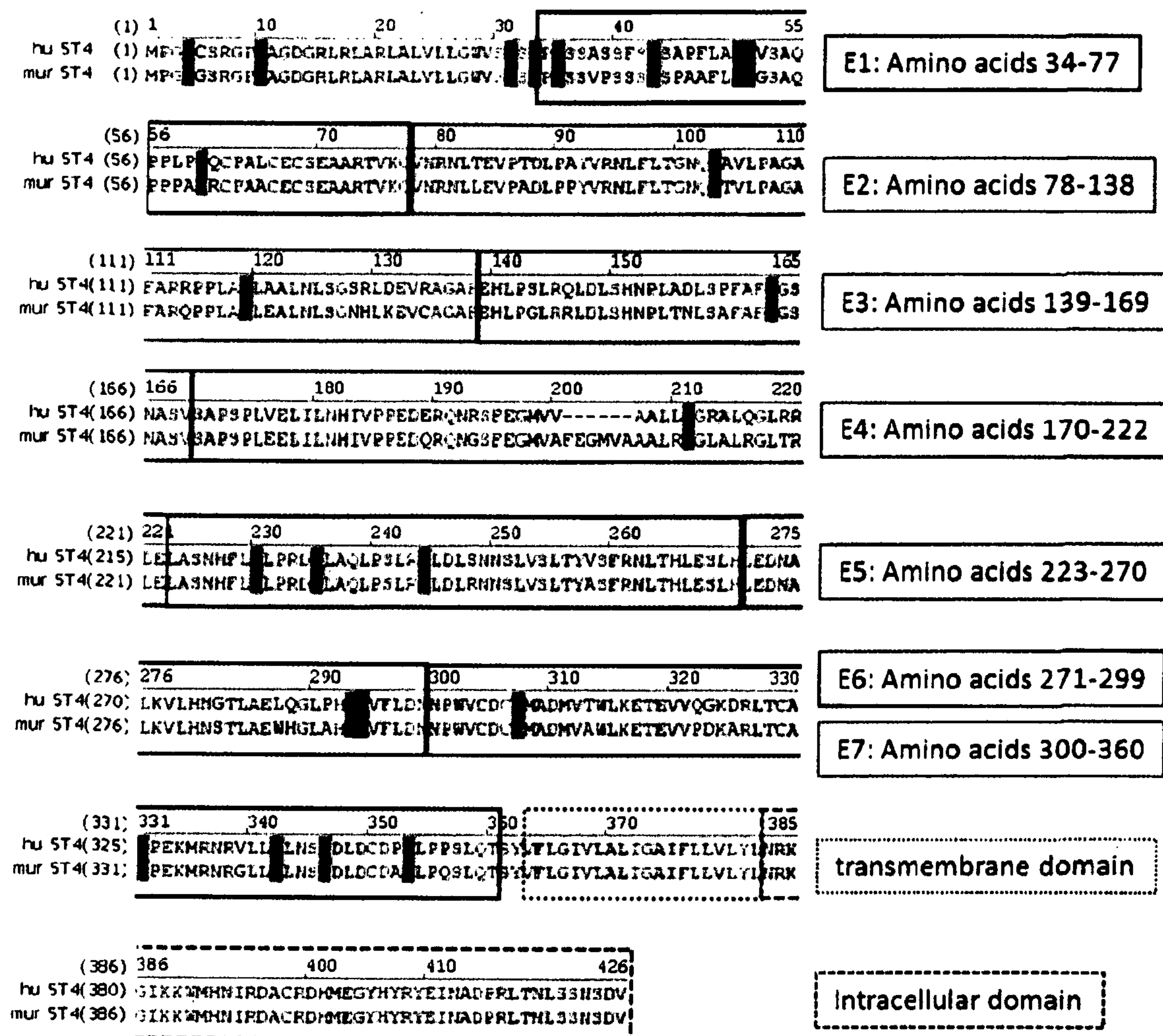


Figure 4

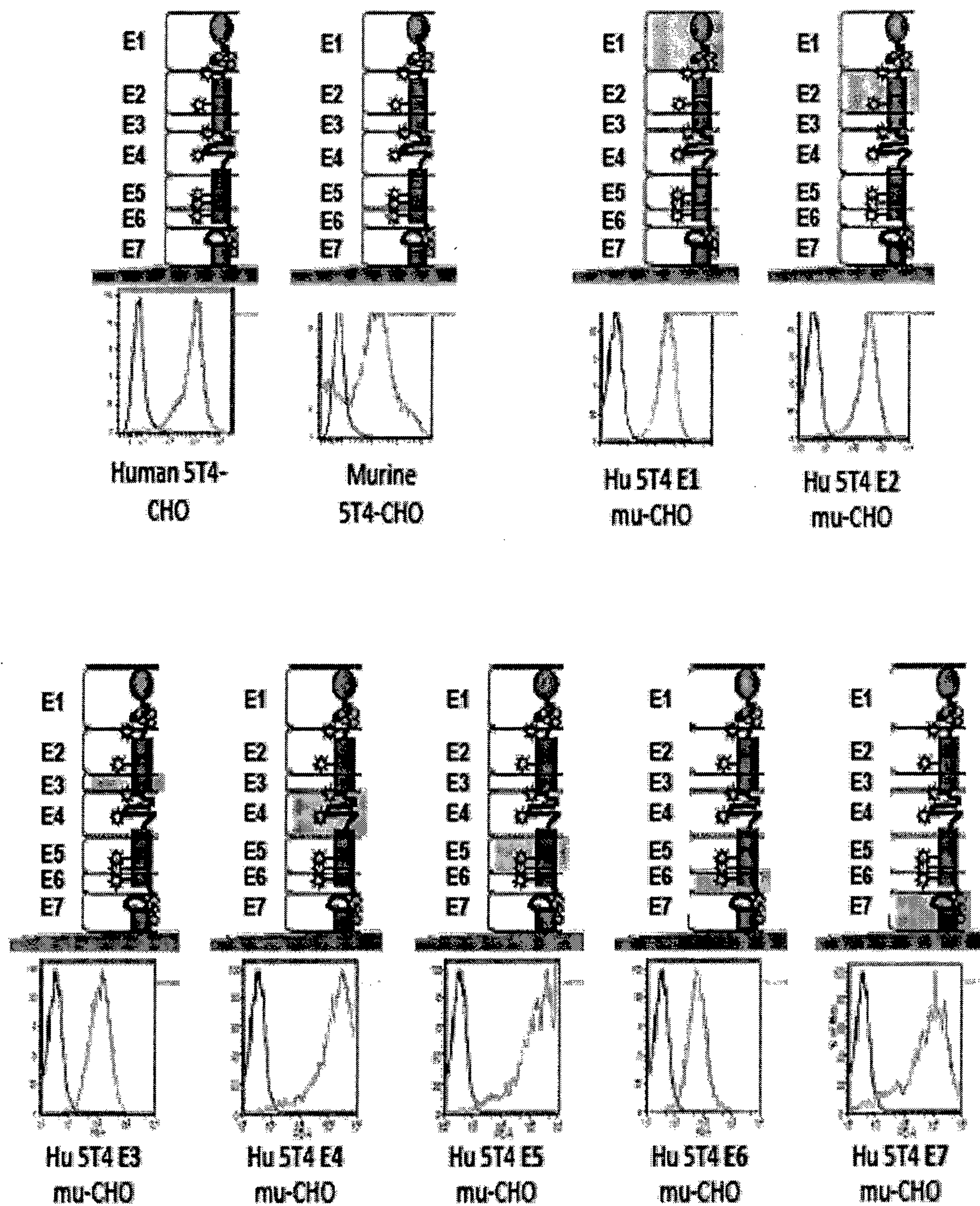


Figure 5(1)

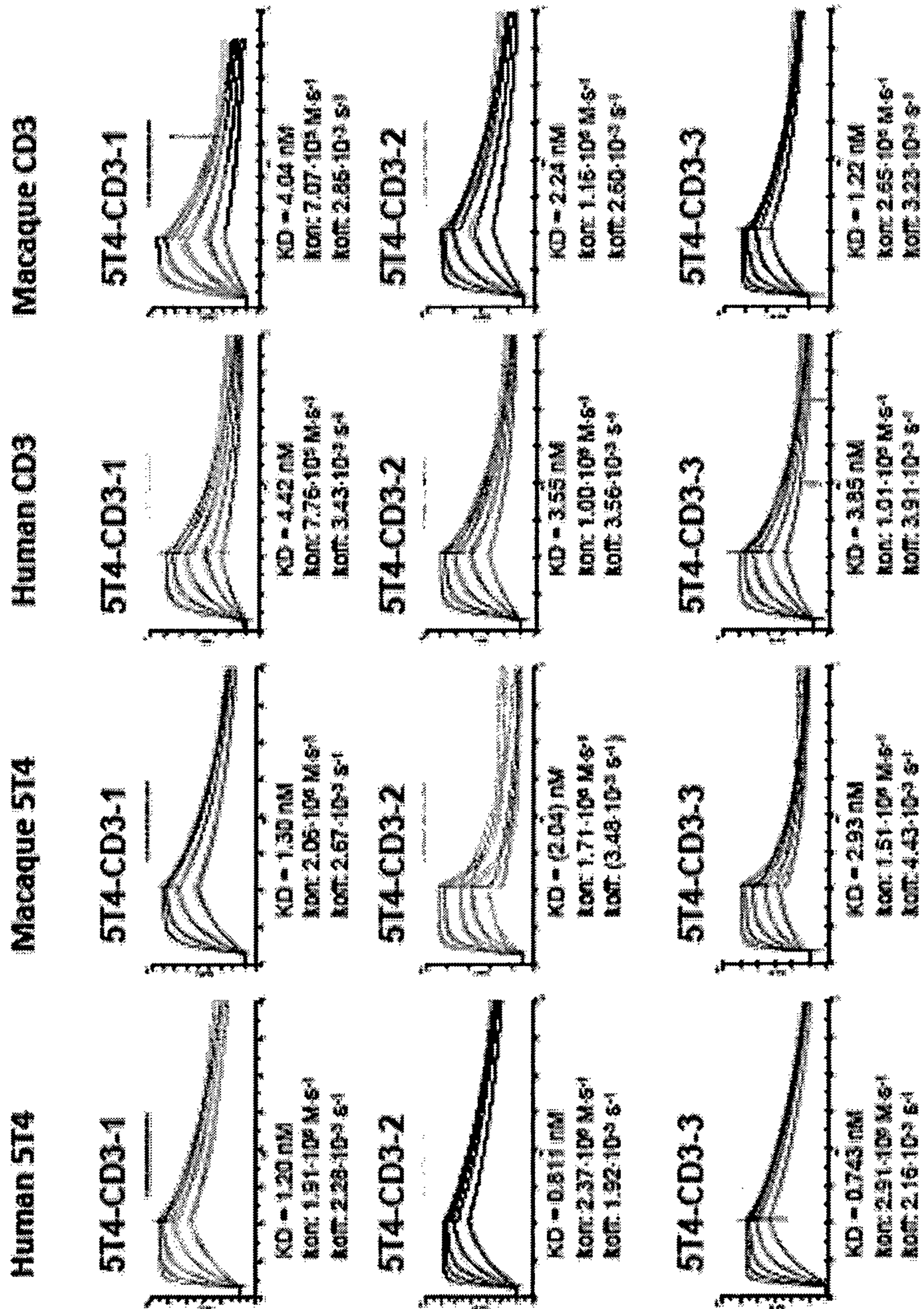


Figure 5(1) (cont'd)

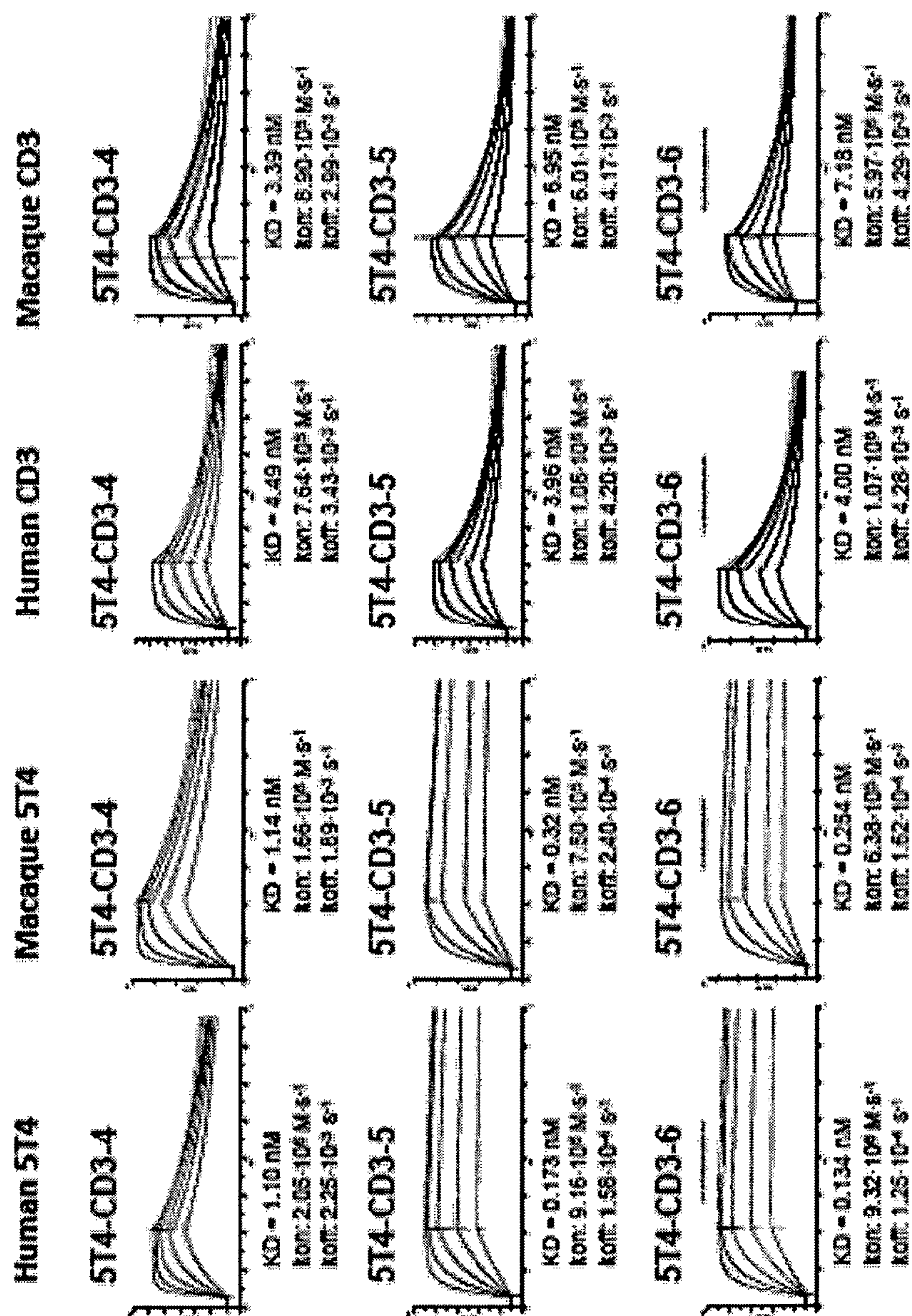


Figure 5(2)

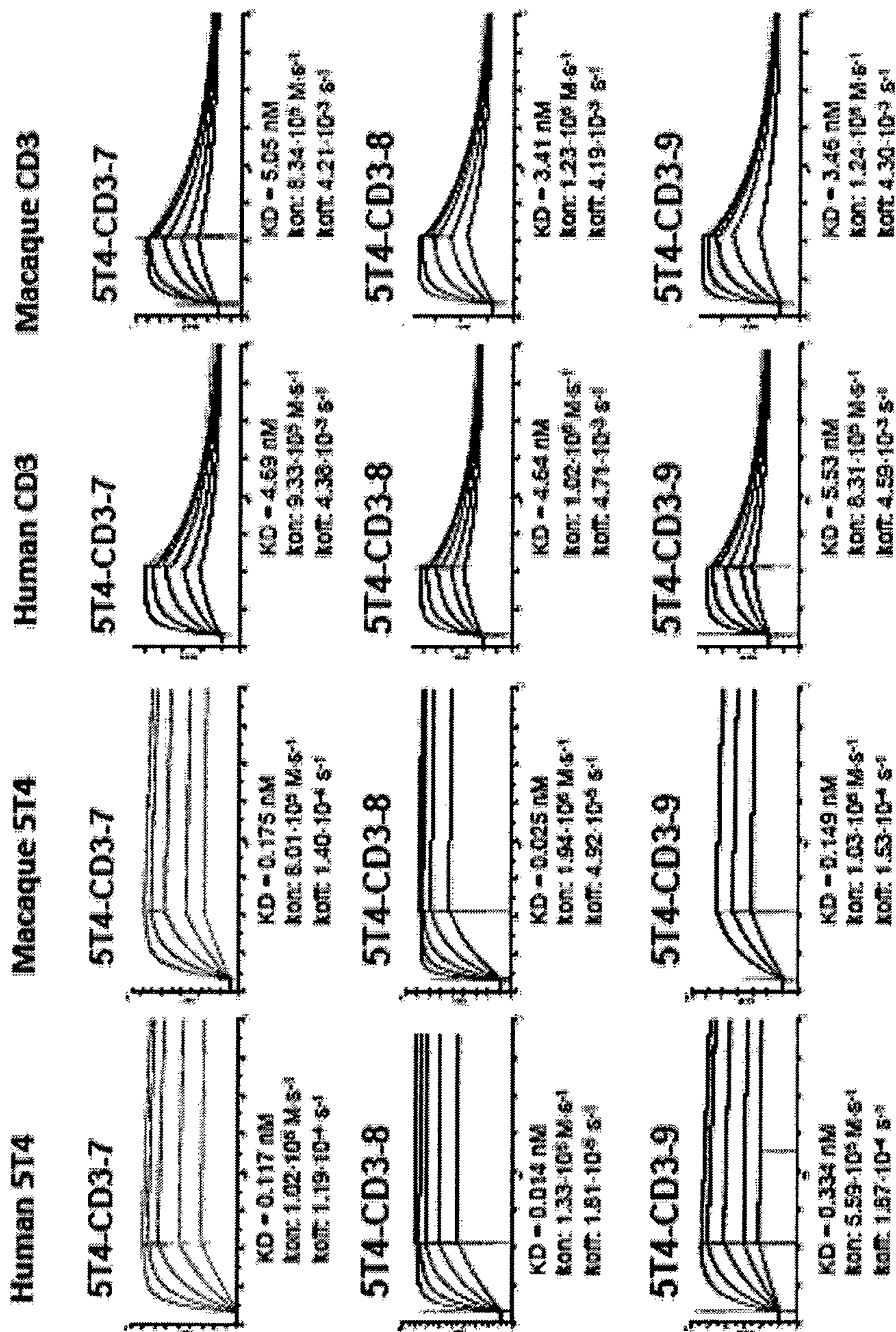


Figure 5(2) (cont'd)

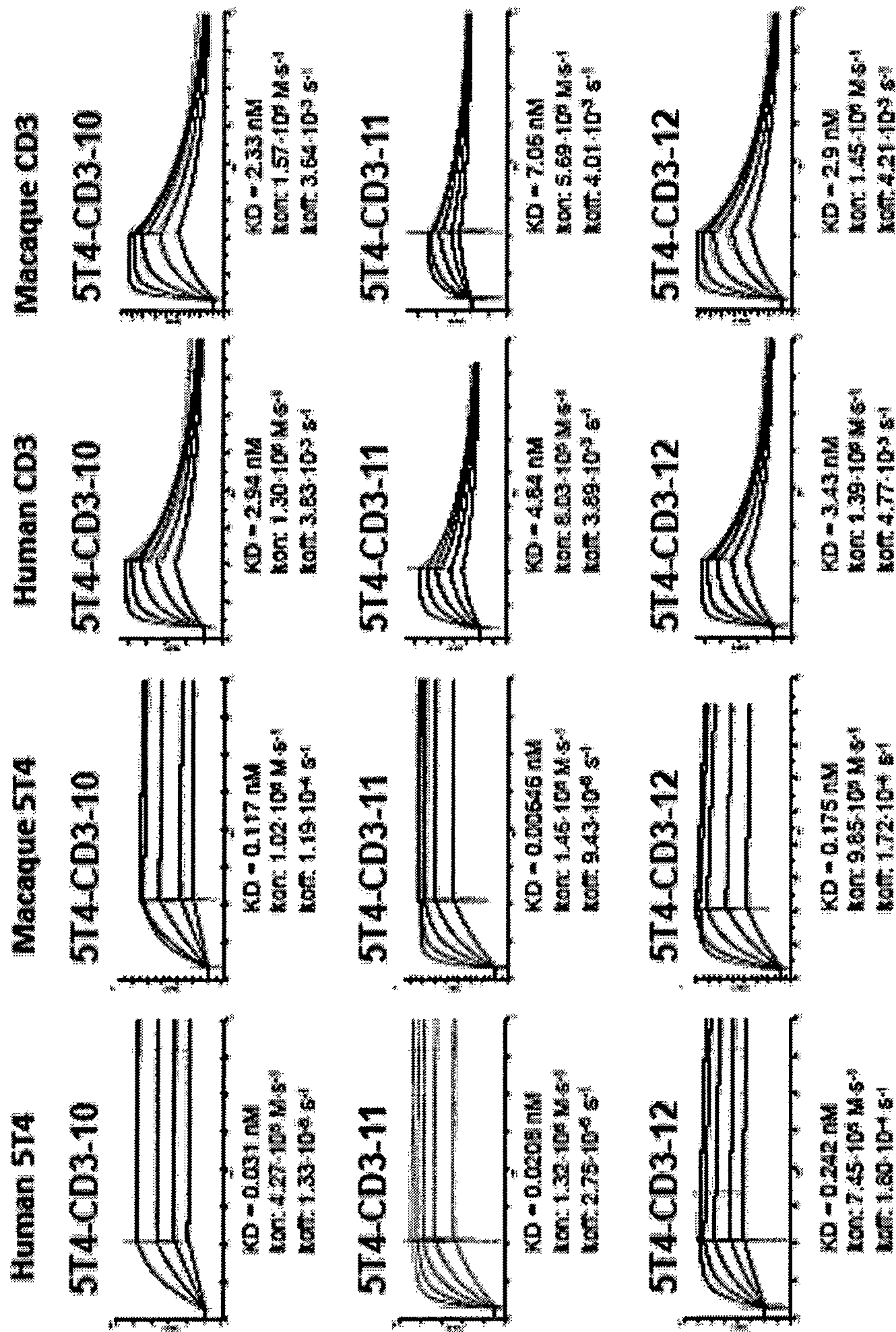


Figure 5(3)

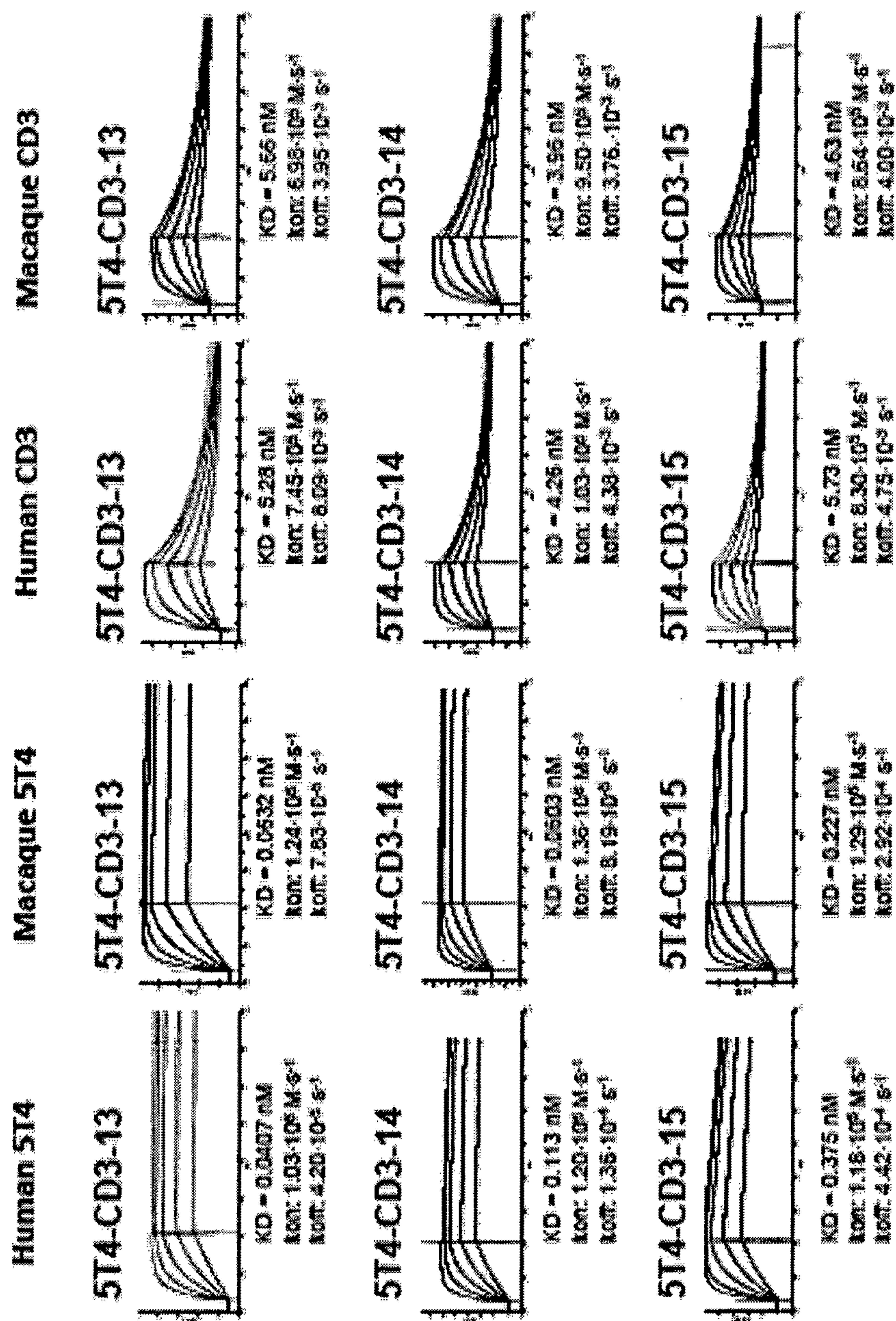


Figure 6

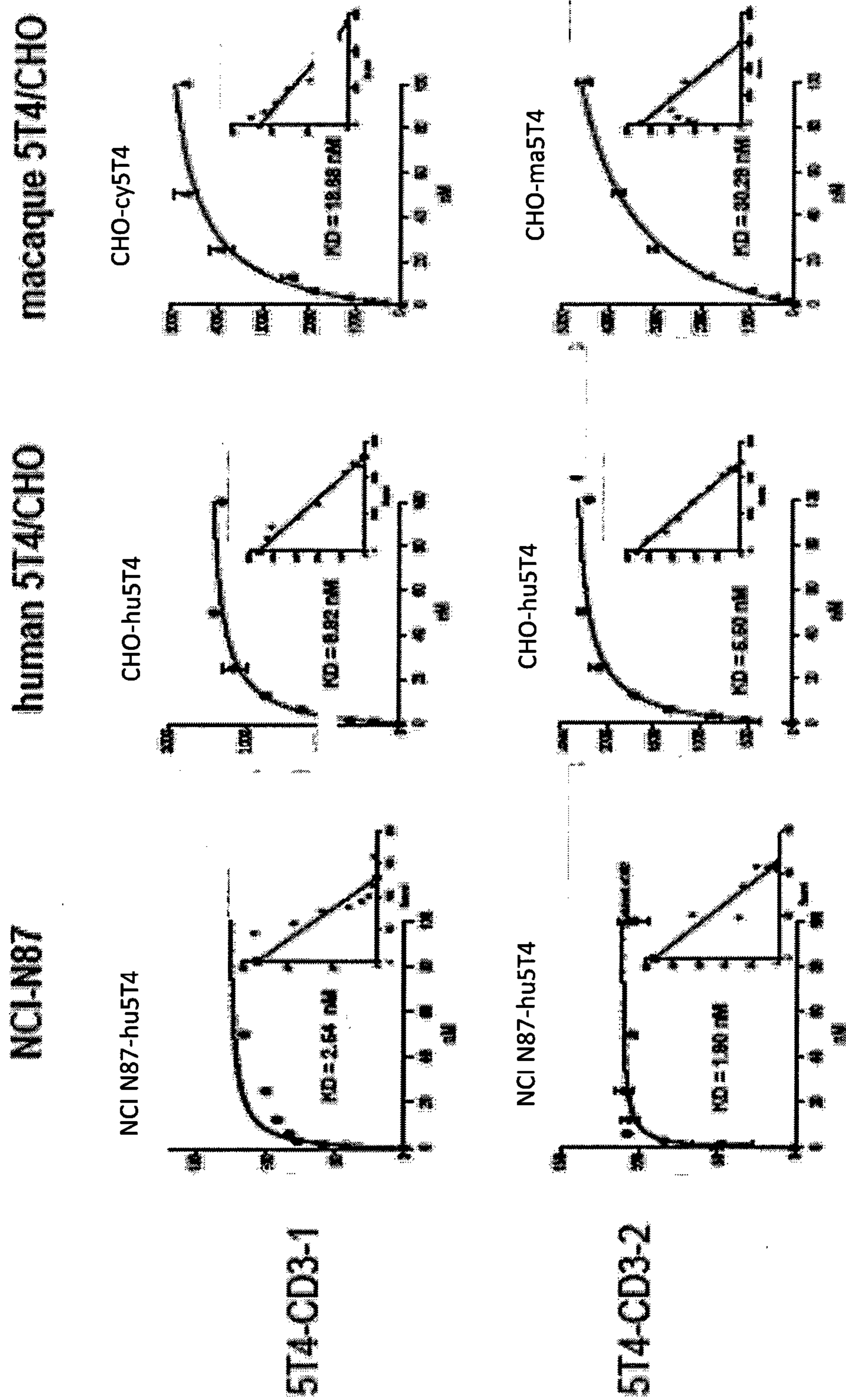


Figure 6 (cont'd)

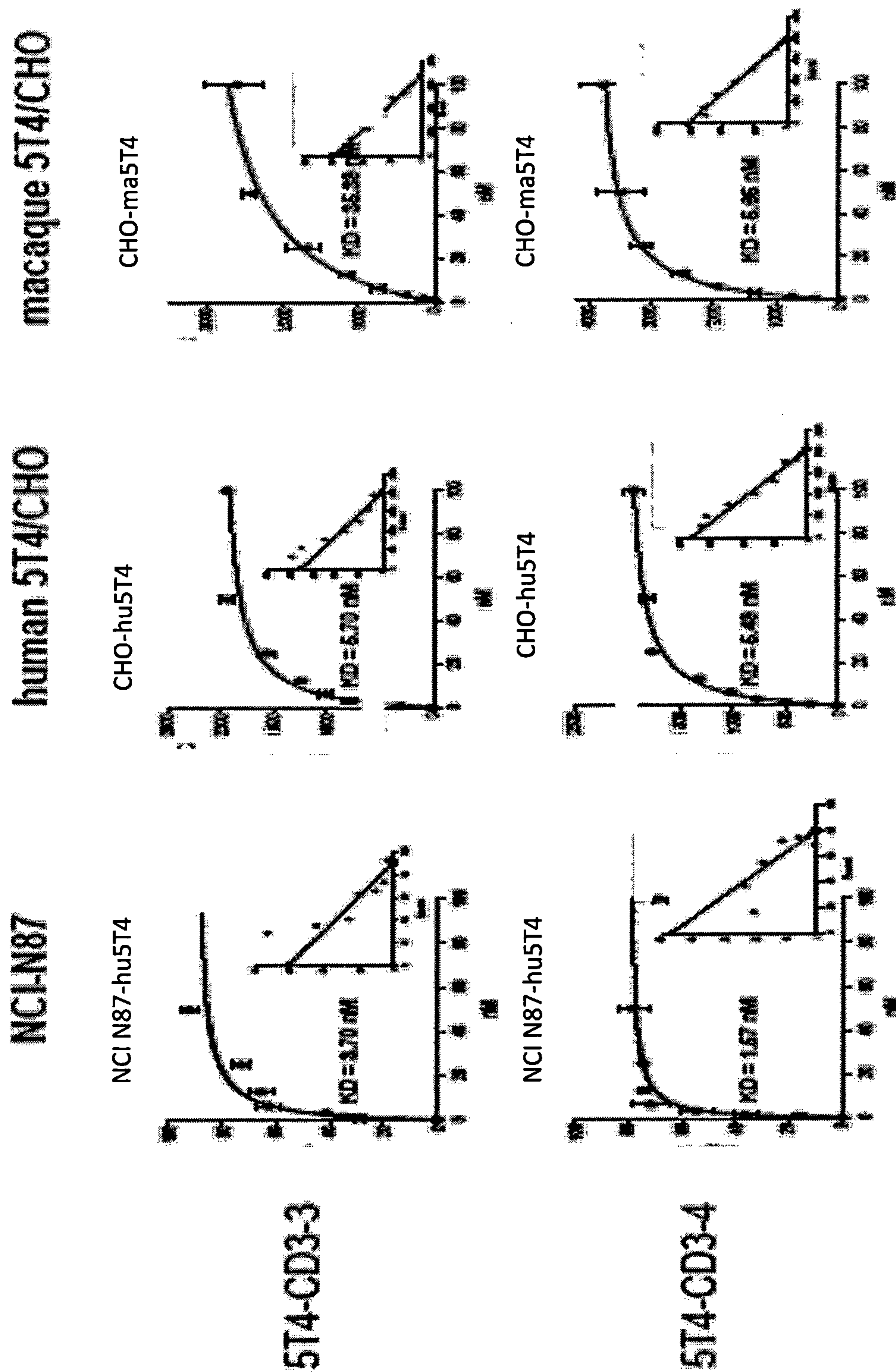


Figure 7

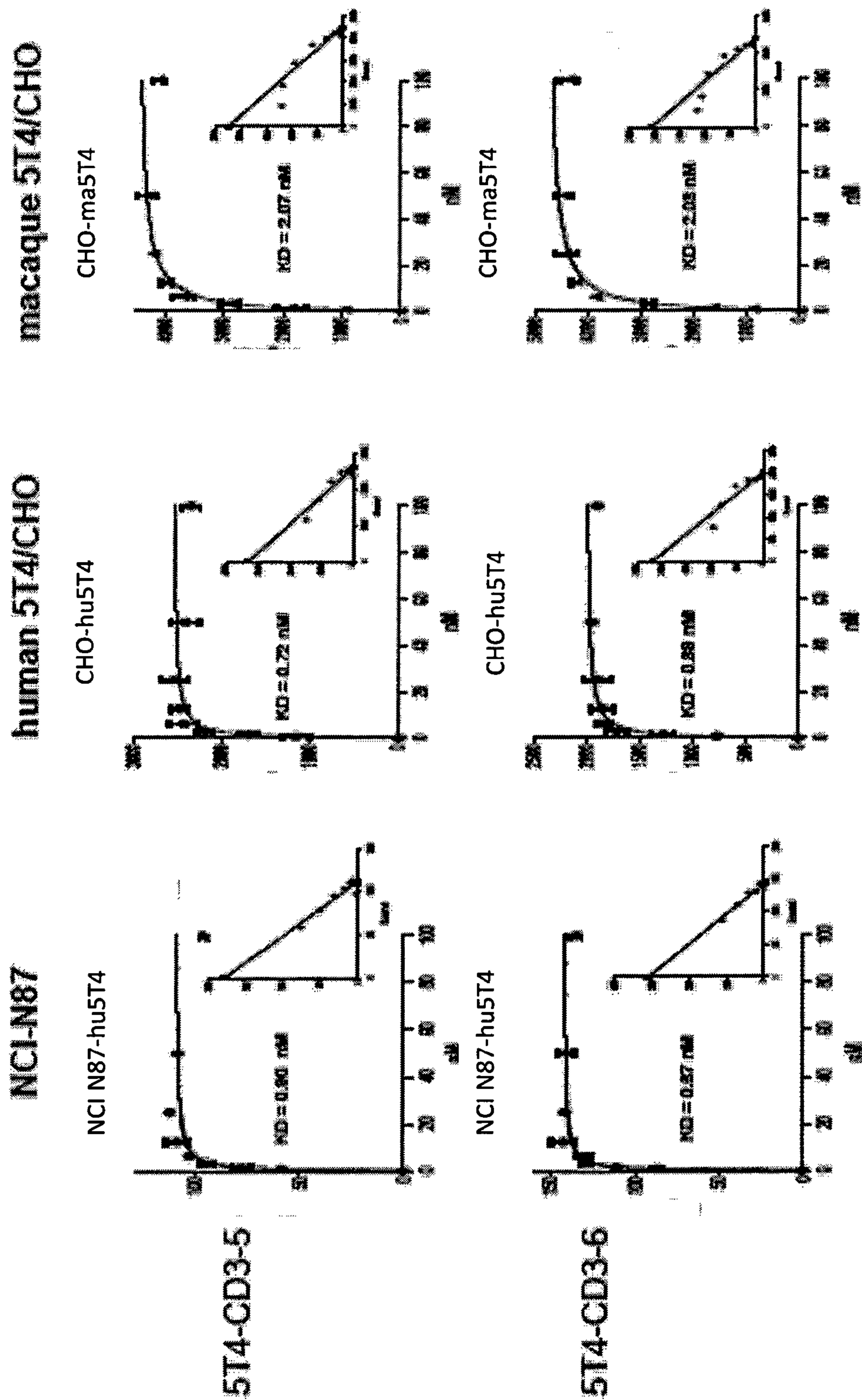


Figure 7 (cont'd)

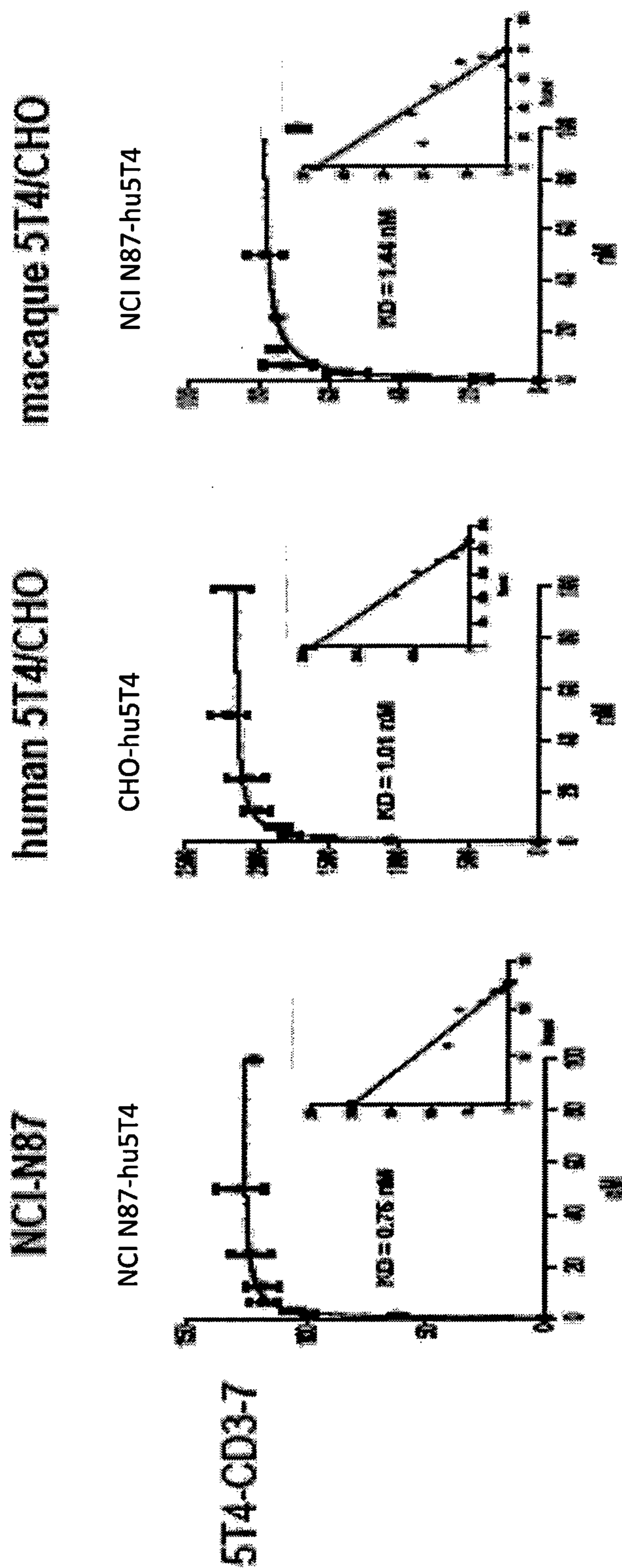


Figure 8(1)

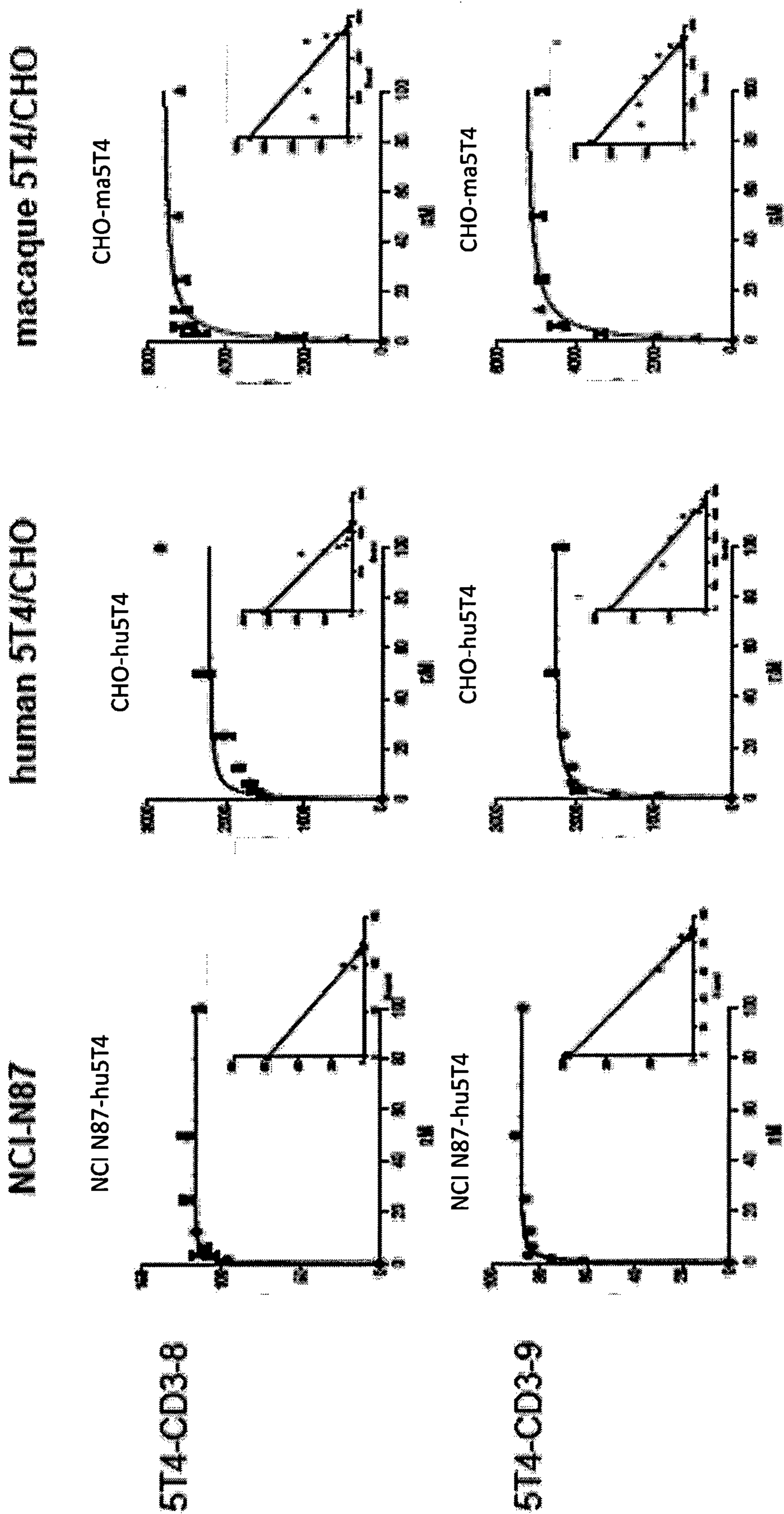


Figure 8(1) (cont'd)

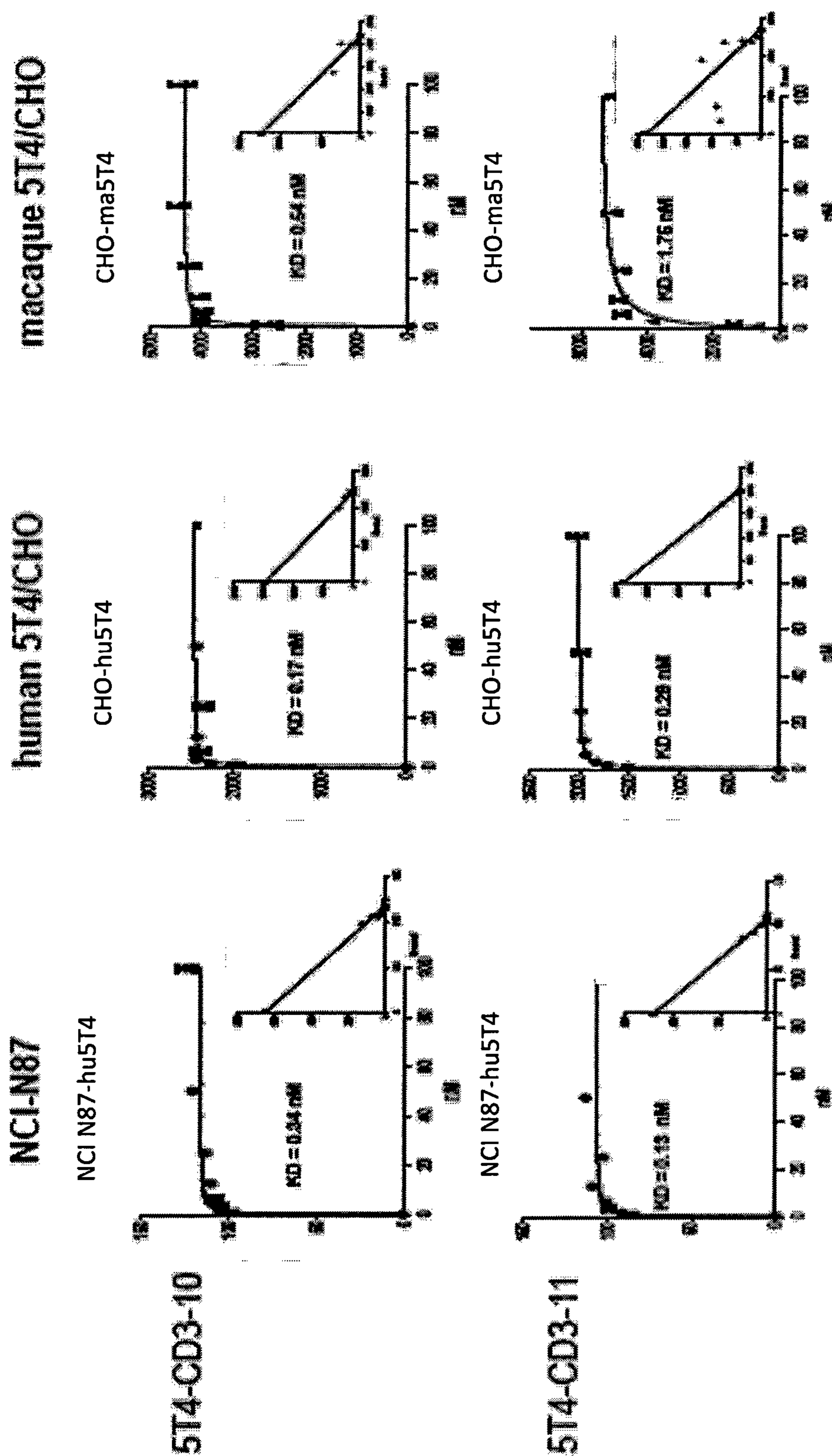


Figure 8(2)

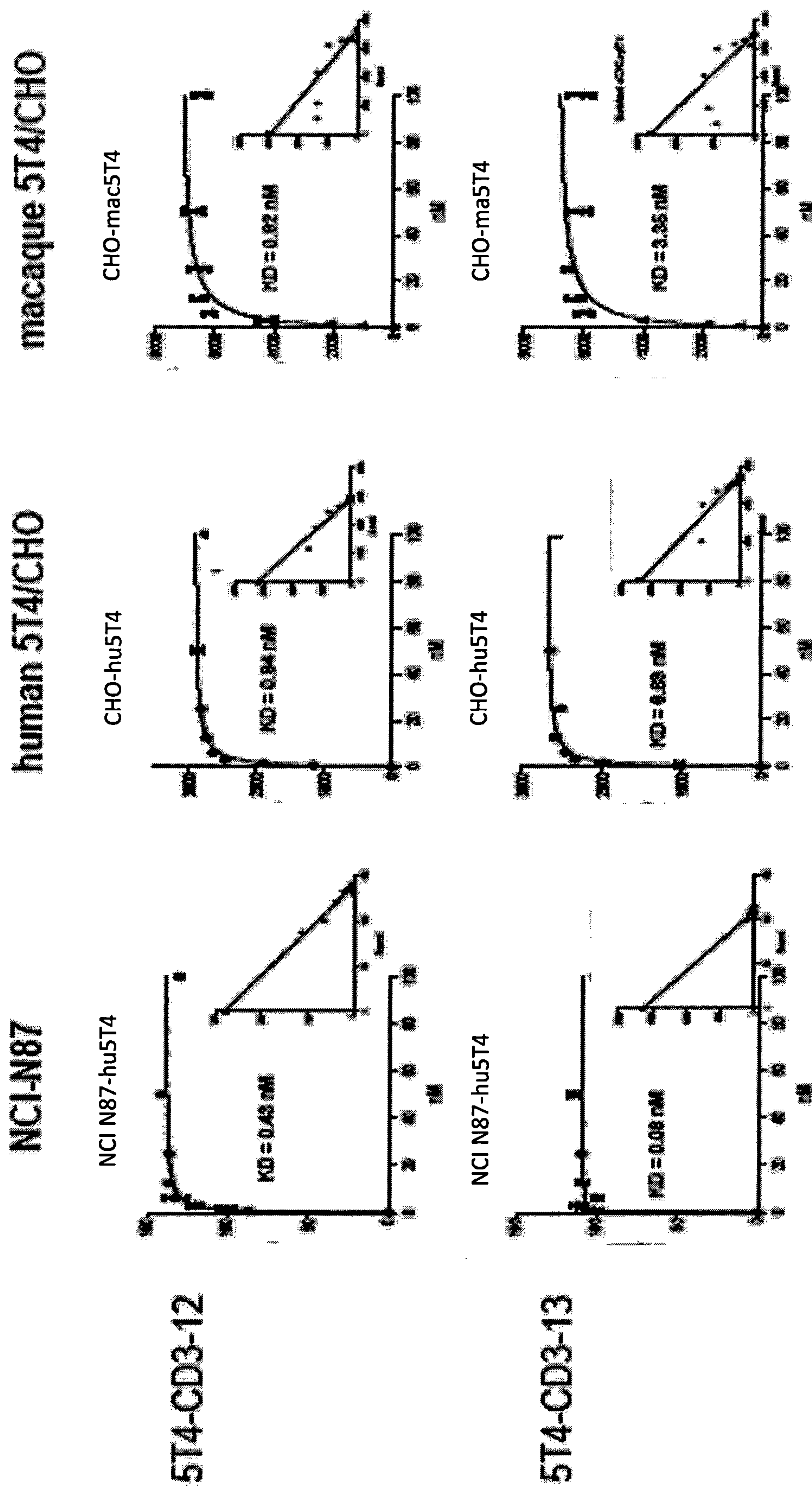


Figure 8(2) (cont'd)

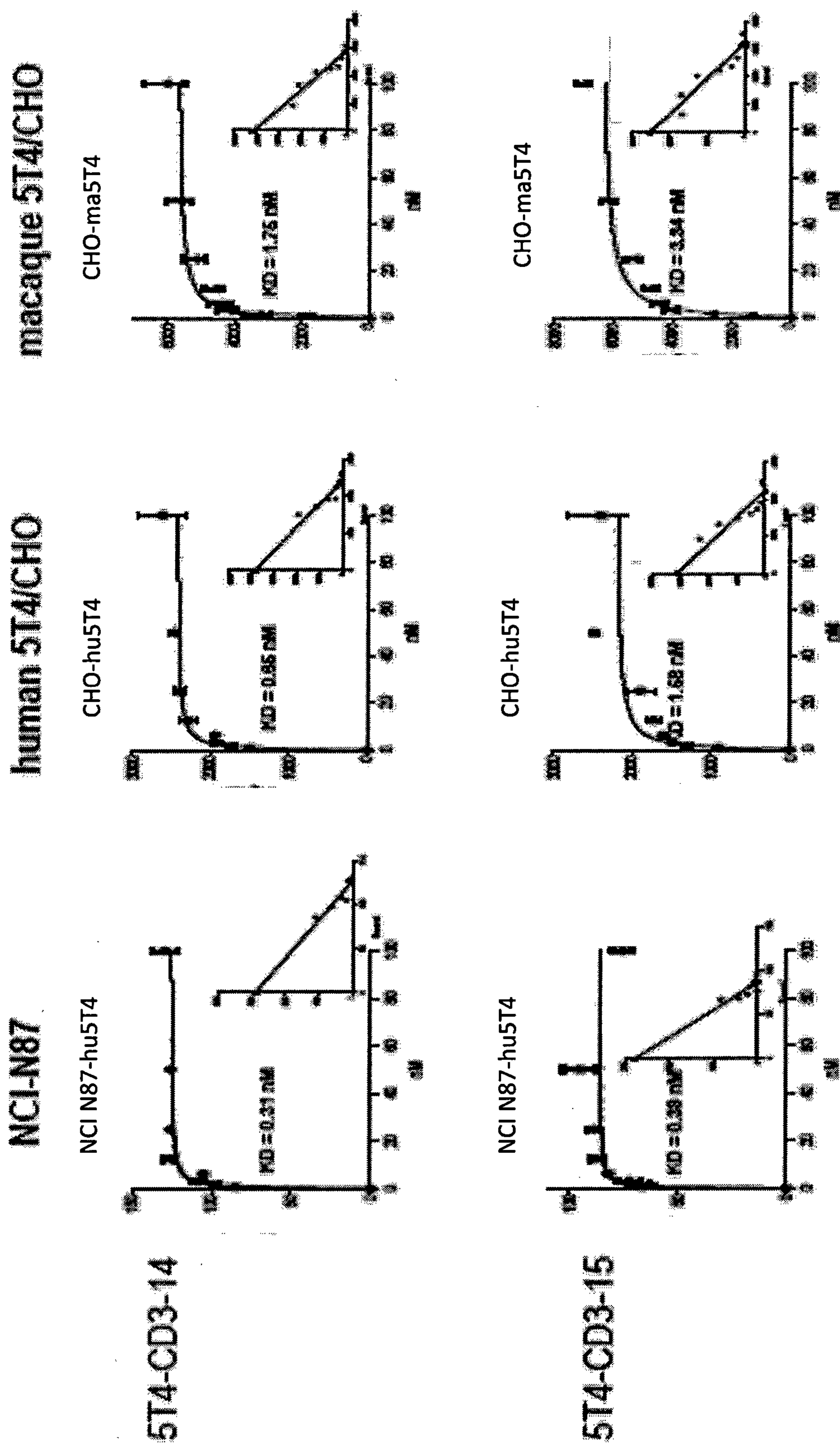
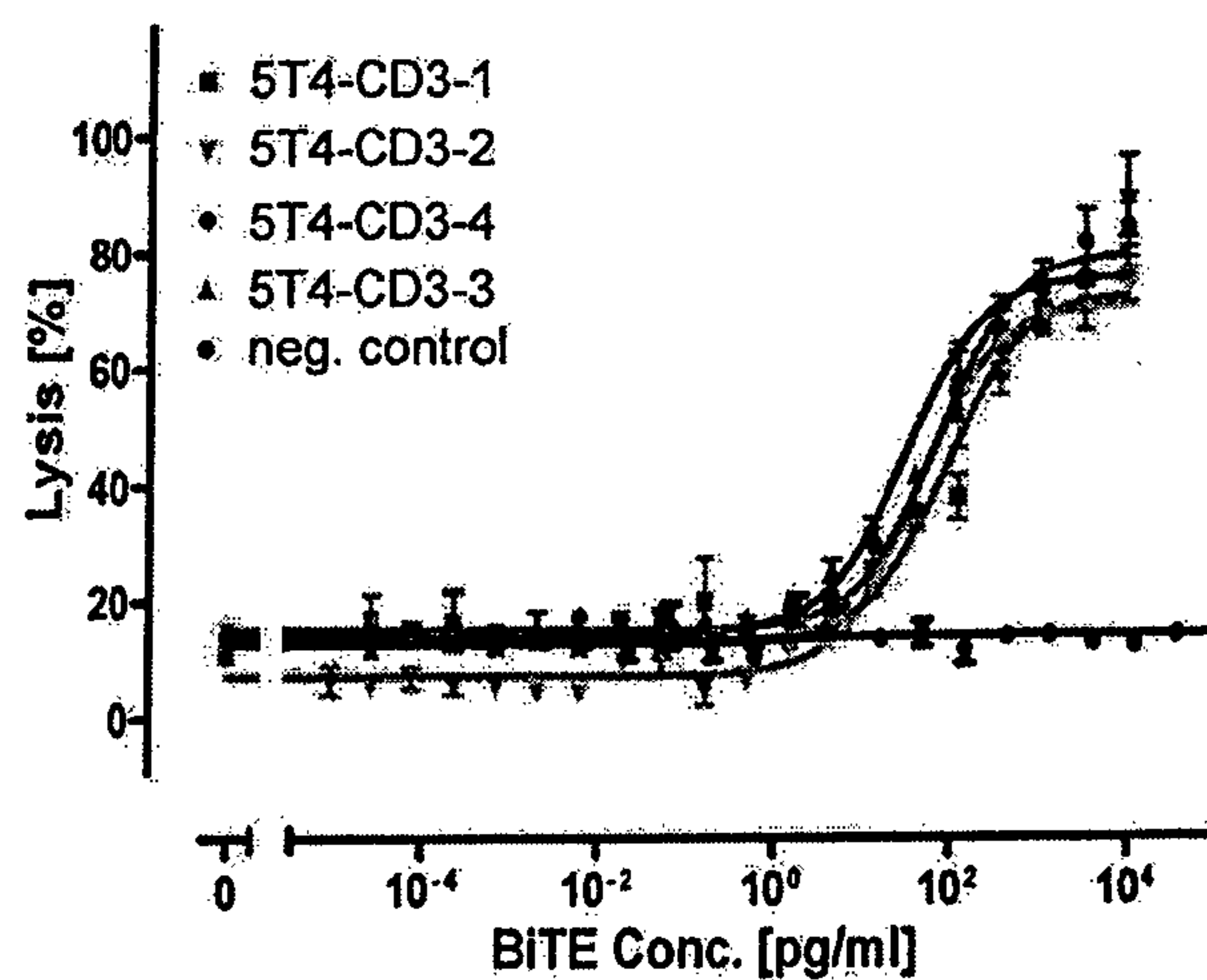


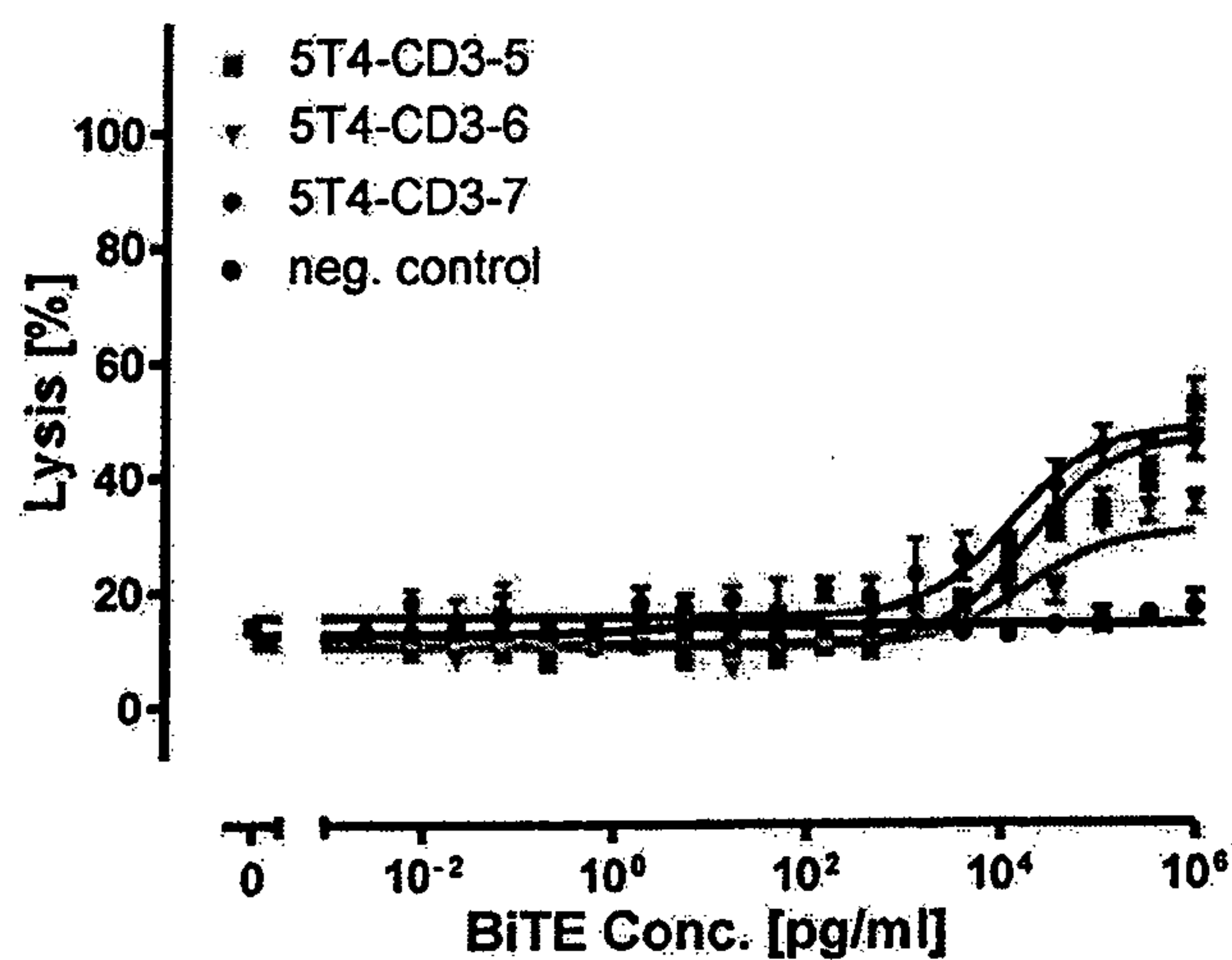
Figure 9(1)

A) Epitope clusters E4



	5T4-CD3-1	5T4-CD3-2	5T4-CD3-4	5T4-CD3-3
EC ₅₀ [pg/mL]	91	43	72	32
R ²	0.95	0.81	0.97	0.95

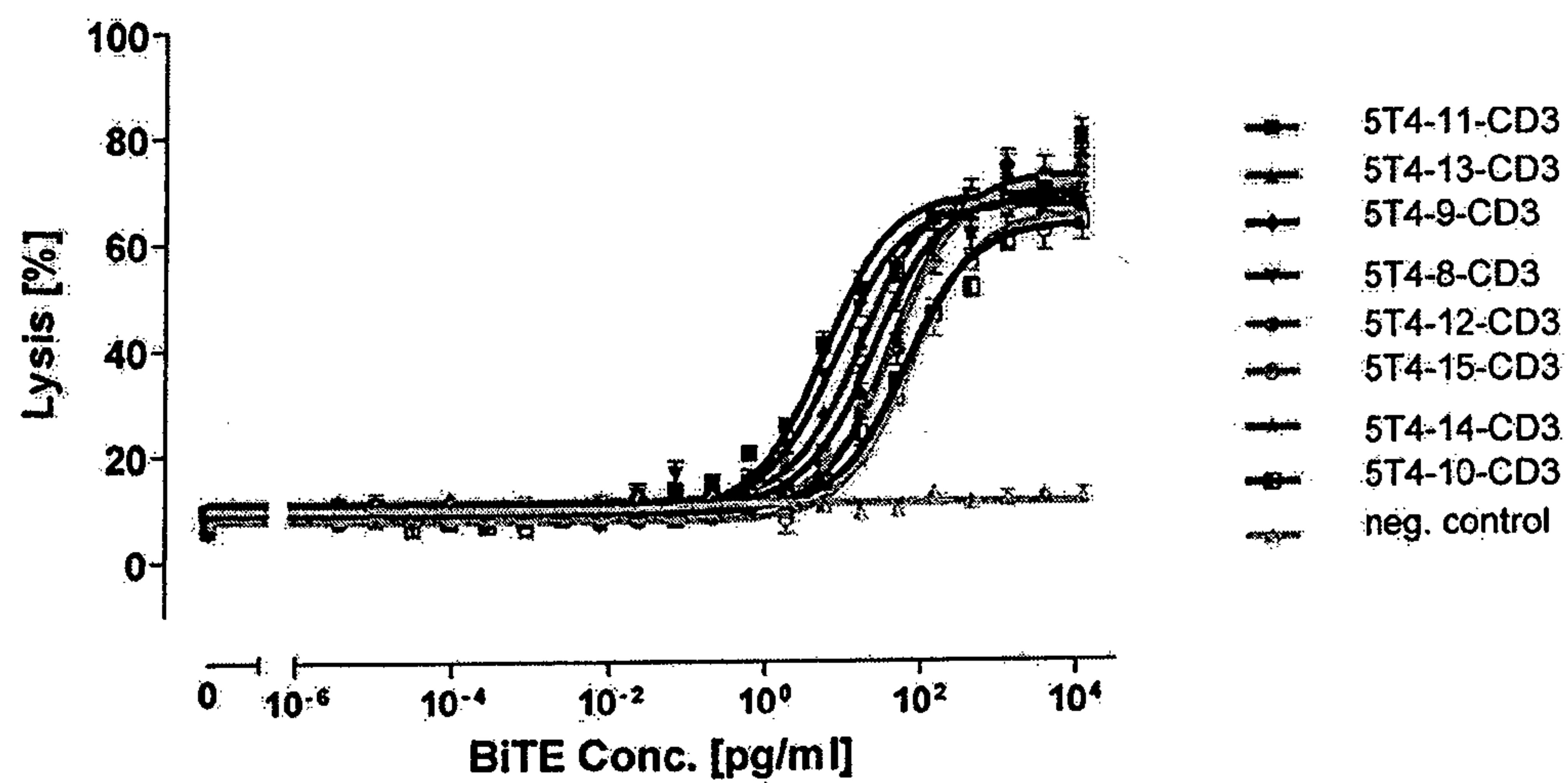
B) Epitope clusters E7



	5T4-CD3-5	5T4-CD3-6	5T4-CD3-7
EC ₅₀ [pg/mL]	20047	17595	13664
R ²	0.84	0.78	0.85

Figure 9(2)

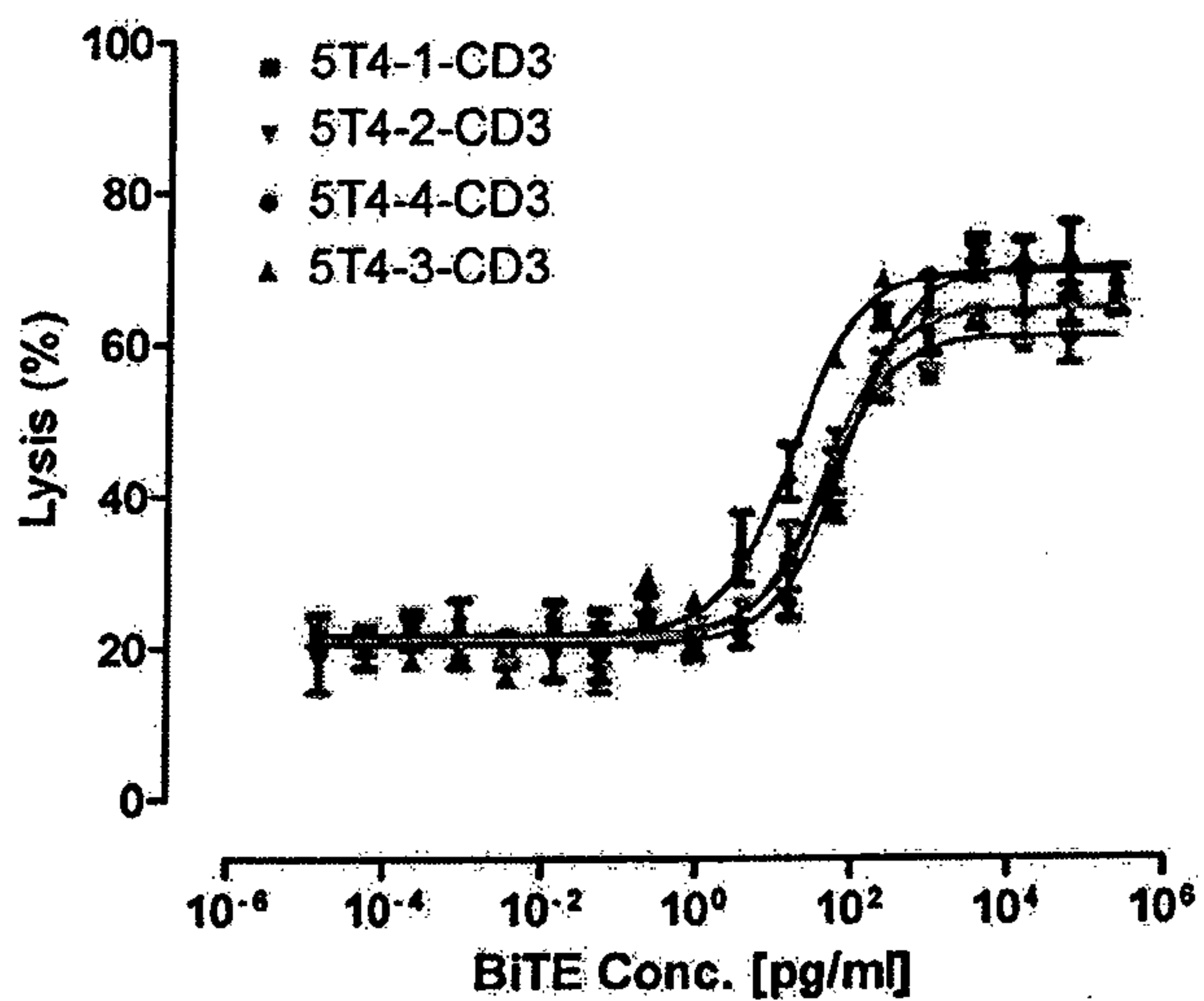
C) Epitope clusters 2/4



	5T4-8- CD3	5T4-9- CD3	5T4-10- CD3	5T4-11- CD3	5T4-12- CD3	5T4-13- CD3	5T4-14- CD3	5T4-15- CD3
EC ₅₀ [pg/mL]	8.31	44.9	64.8	5.89	47.2	17.1	24.5	76.0
R ²	0.9778	0.9732	0.9730	0.9636	0.9798	0.9726	0.9762	0.9659

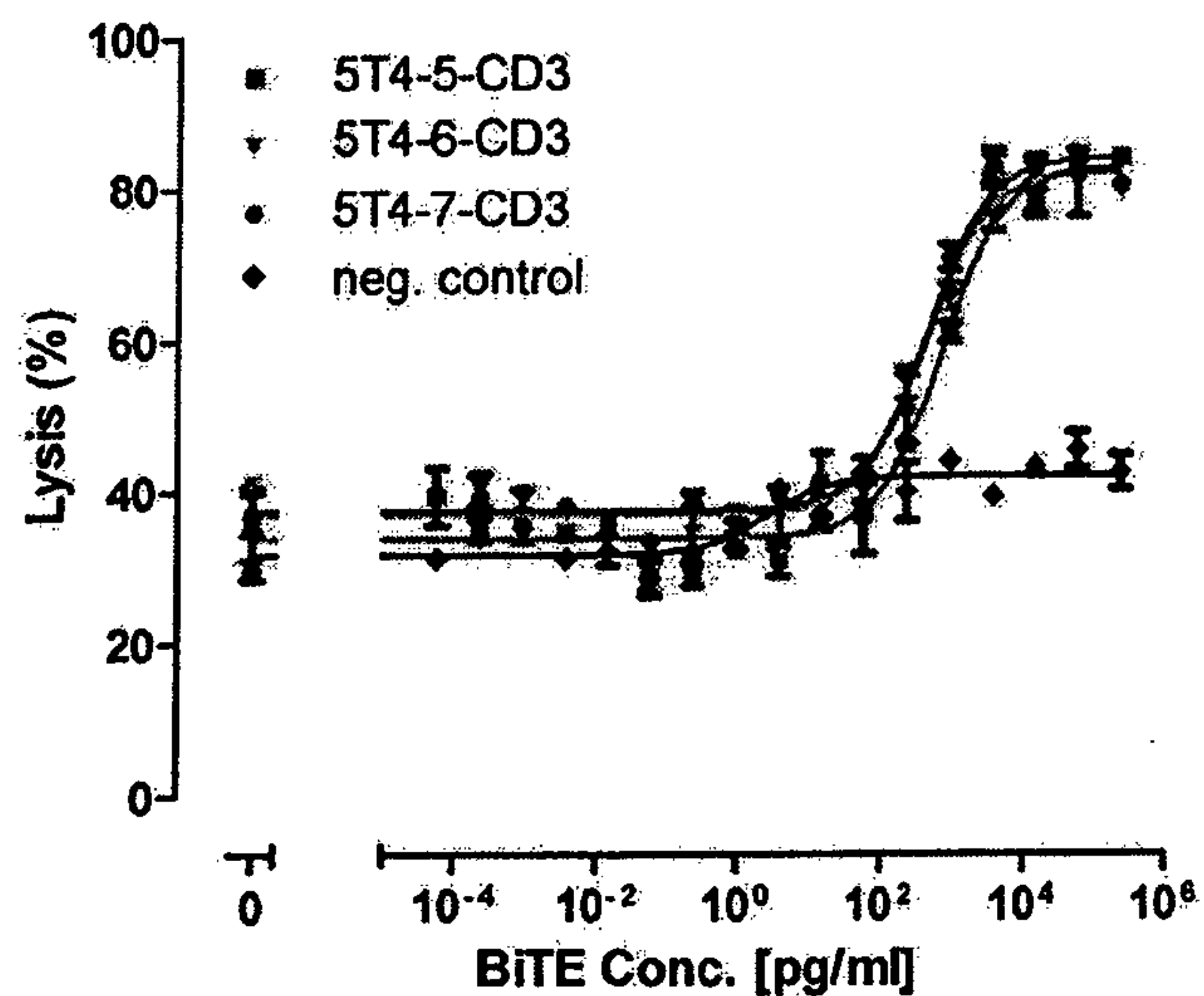
Figure 10(1)

A) Epitope clusters E4



	5T4-1-CD3	5T4-2-CD3	5T4-4-CD3	5T4-3-CD3
EC ₅₀ [pg/mL]	48.3	47.17	81.99	16.89
R ²	0.97	0.97	0.98	0.97

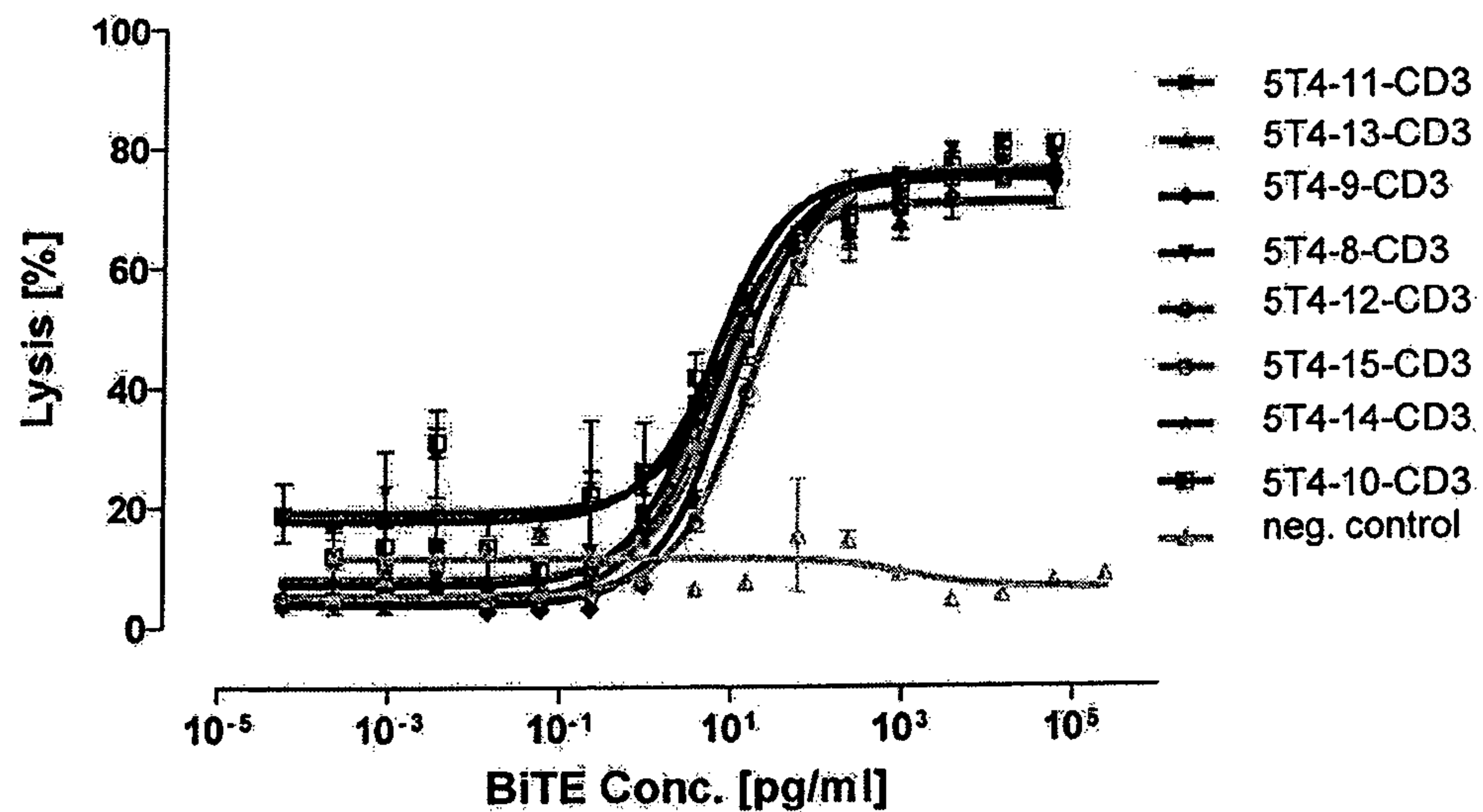
B) Epitope clusters E7



	5T4-5-CD3	5T4-6-CD3	5T4-7-CD3
EC ₅₀ [pg/mL]	746	444.5	463.1
R ²	0.96	0.95	0.98

Figure 10(2)

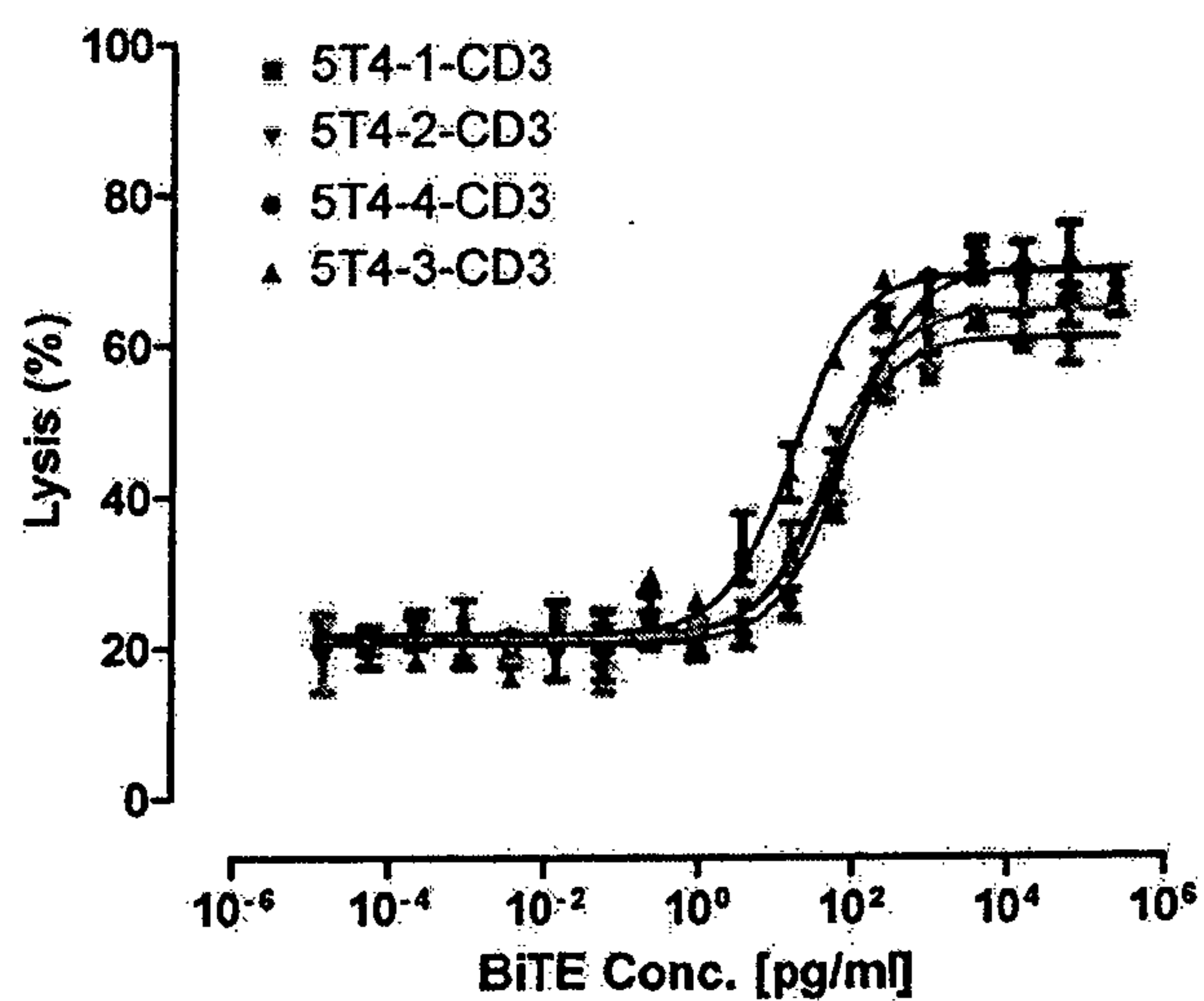
C) Epitope clusters 2/4



	5T4-8- CD3	5T4-9- CD3	5T4-10- CD3	5T4-11- CD3	5T4-12- CD3	5T4-13- CD3	5T4-14- CD3	5T4-15- CD3
EC ₅₀ pg/mL]	5.07	9.56	7.08	7.04	9.16	5.13	10.2	17.1
R ²	0.9867	0.9926	0.9338	0.9708	0.9806	0.9834	0.9487	0.9940

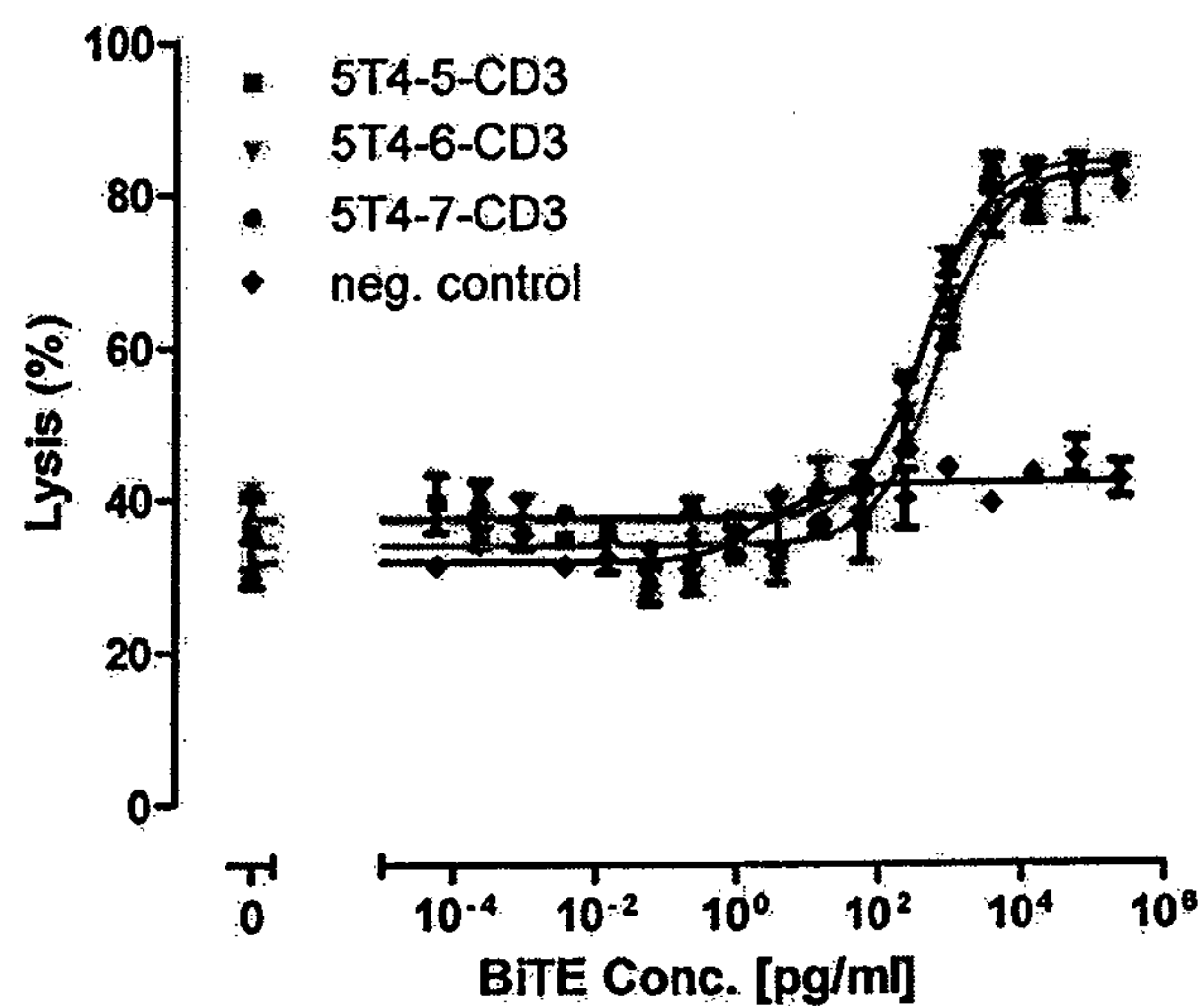
Figure 11(1)

A) Epitope clusters E4



	5T4-1-CD3	5T4-2-CD3	5T4-4-CD3	5T4-3-CD3
EC ₅₀ [pg/mL]	7.46	1.55	16.95	4.613
R ²	0.99	0.99	0.99	0.98

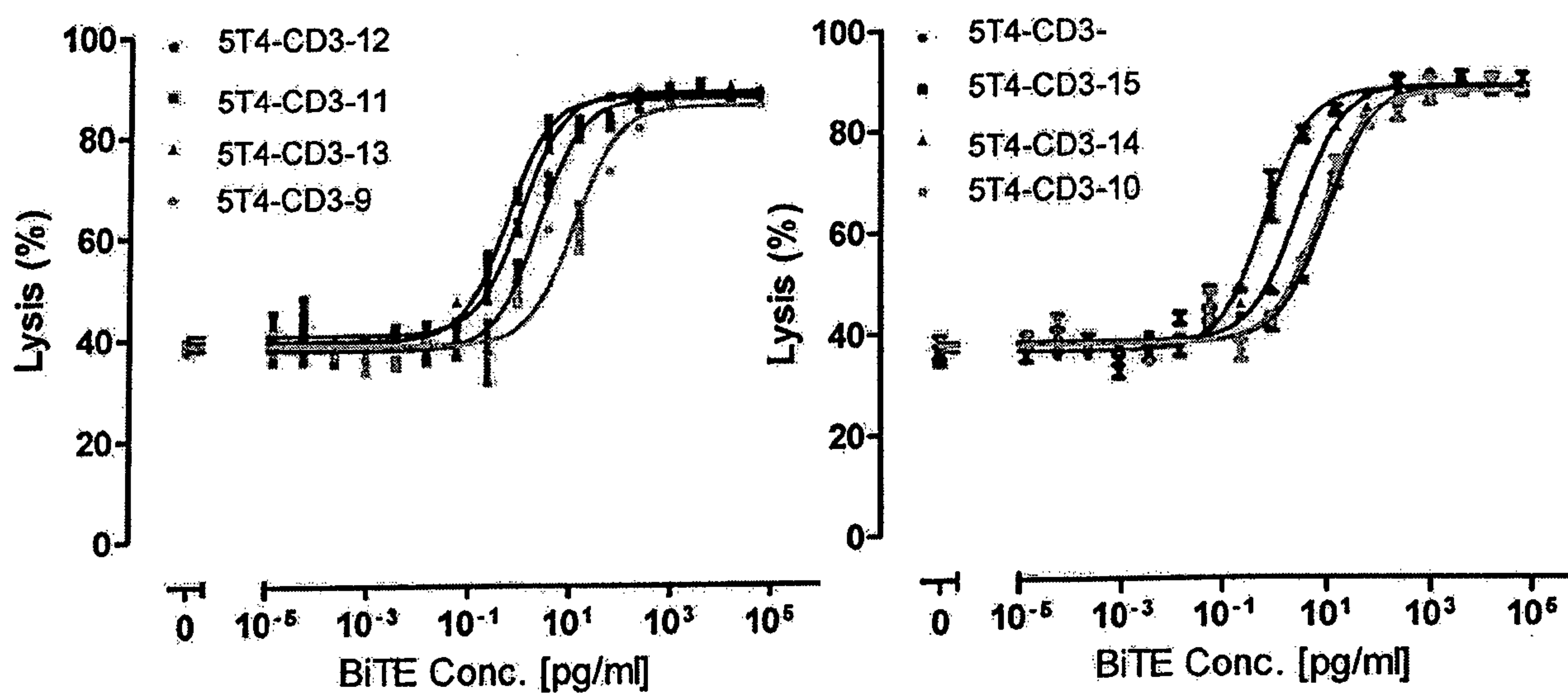
B) Epitope clusters E7



	5T4-5-CD3	5T4-6-CD3	5T4-7-CD3
EC ₅₀ [pg/mL]	5278	7550	7567
R ²	0.98	0.96	0.97

Figure 11(2)

C) Epitope clusters 2/4

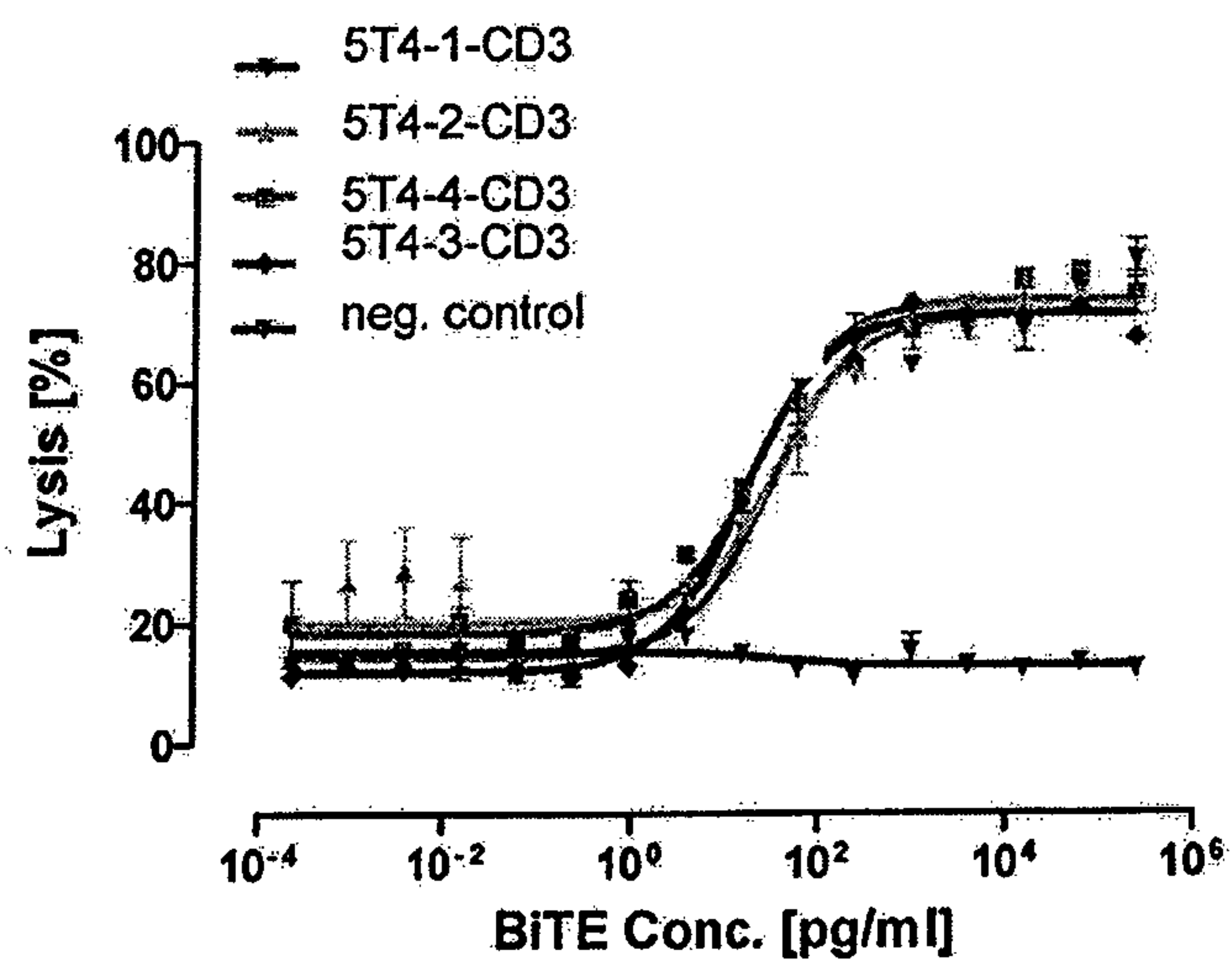


	5T4-CD3-8	5T4-CD3-15	5T4-CD3-14	5T4-CD3-10
EC ₅₀ [pg/mL]	0.7	9.76	2.84	7.42
R ²	0.99	0.99	0.98	0.98

	5T4-CD3-12	5T4-CD3-11	5T4-CD3-13	5T4-CD3-9
EC ₅₀ [pg/mL]	2.41	0.56	1.03	11.65
R ²	0.98	0.98	0.98	0.97

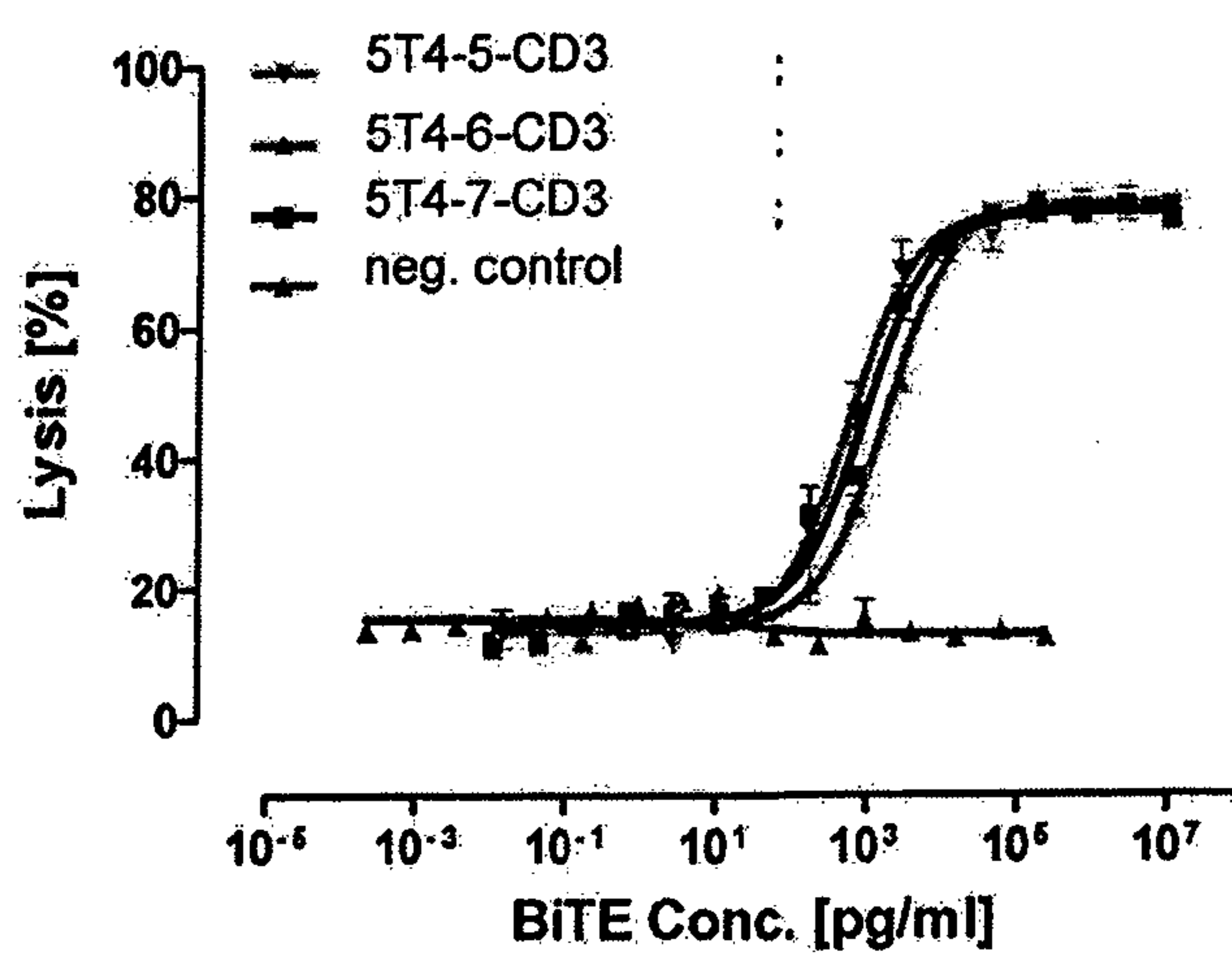
Figure 12(1)

A) Epitope clusters E4



	5T4-1-CD3	5T4-2-CD3	5T4-3-CD3	5T4-4-CD3
EC ₅₀ [pg/mL]	31.2	40.0	16.5	21.9
R ²	0.97	0.94	0.99	0.97

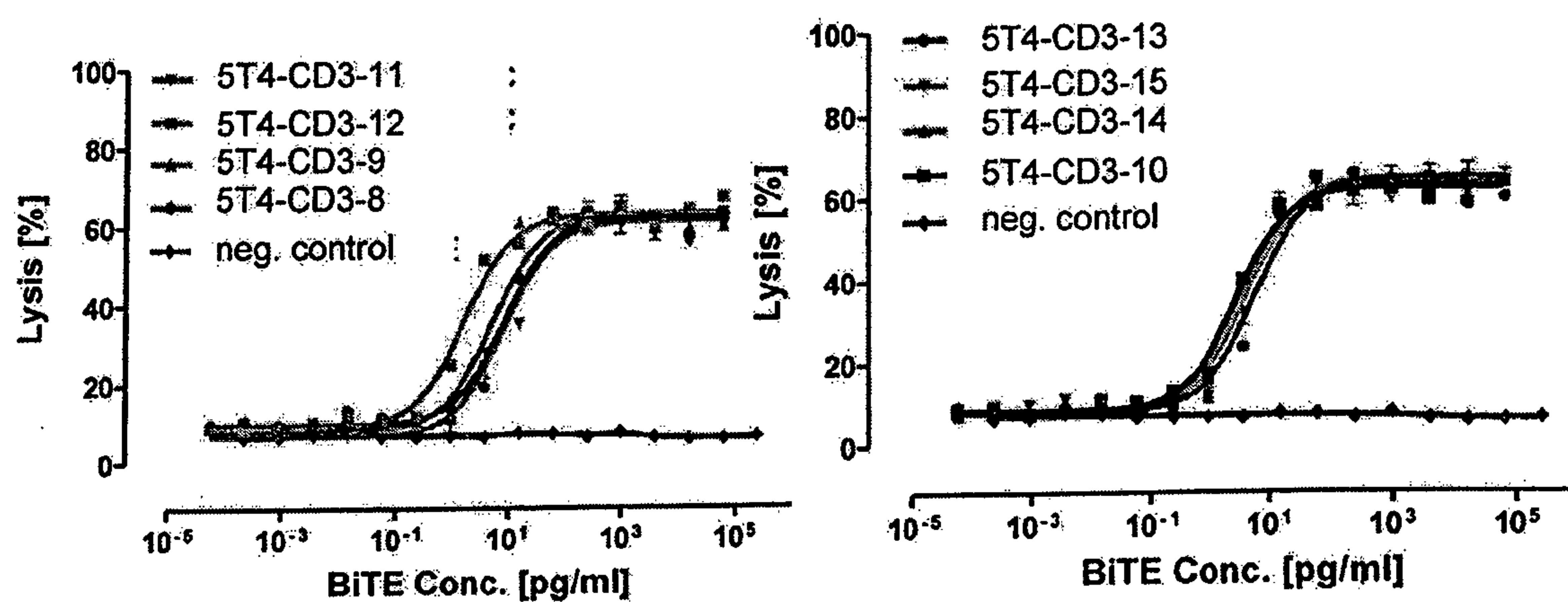
B) Epitope clusters E7



	5T4-5-CD3	5T4-6-CD3	5T4-7-CD3
EC ₅₀ [pg/mL]	616	1850	959
R ²	0.99	0.99	0.99

Figure 12(2)

C) Epitope clusters 2/4



	5T4-CD3-8	5T4-CD3-9	5T4-CD3-10	5T4-CD3-11
EC ₅₀ [pg/mL]	8.65	5.27	2.95	8.44
R ²	0.9797	0.9664	0.9907	0.9800

	5T4-CD3-12	5T4-CD3-13	5T4-CD3-14	5T4-CD3-15
EC ₅₀ [pg/mL]	1.65	6.02	4.33	3.92
R ²	0.9869	0.9761	0.9903	0.9814

Figure 13

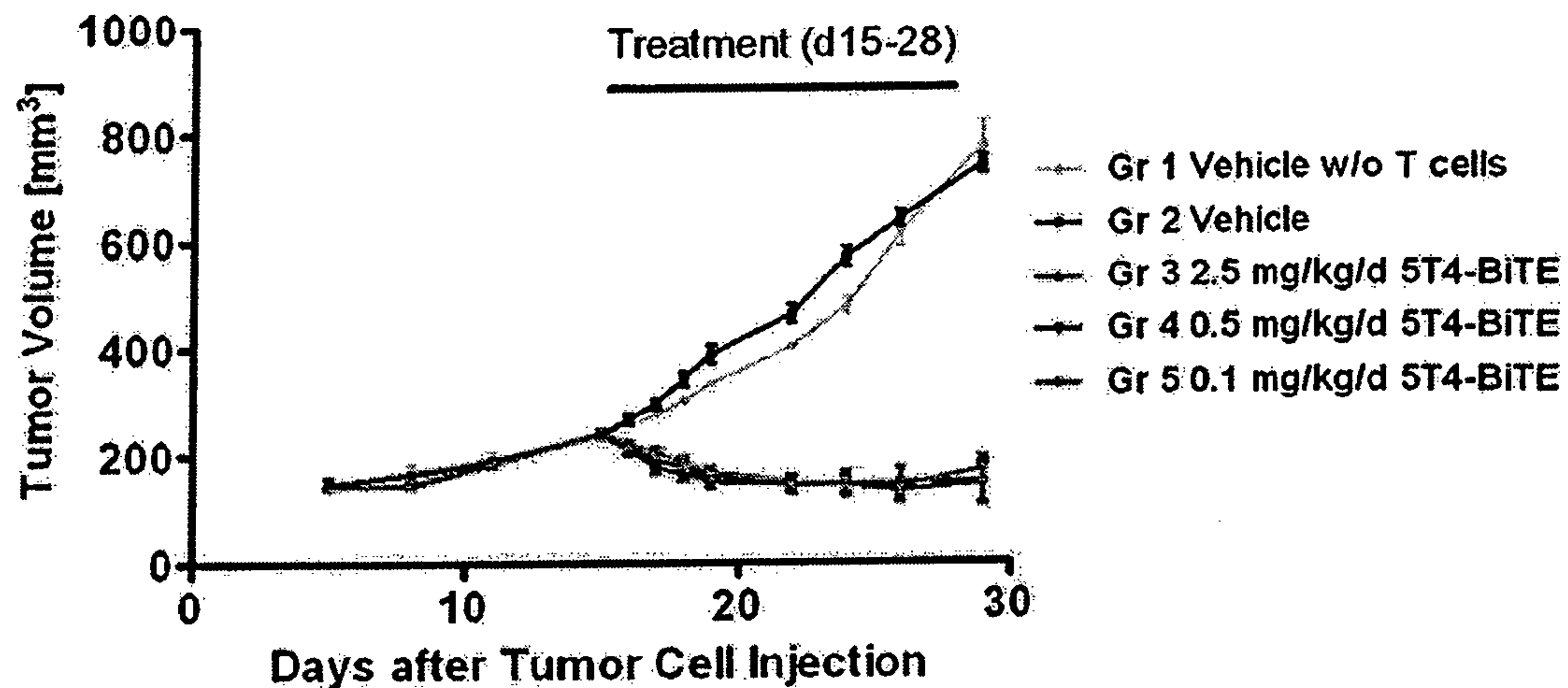


Figure 14

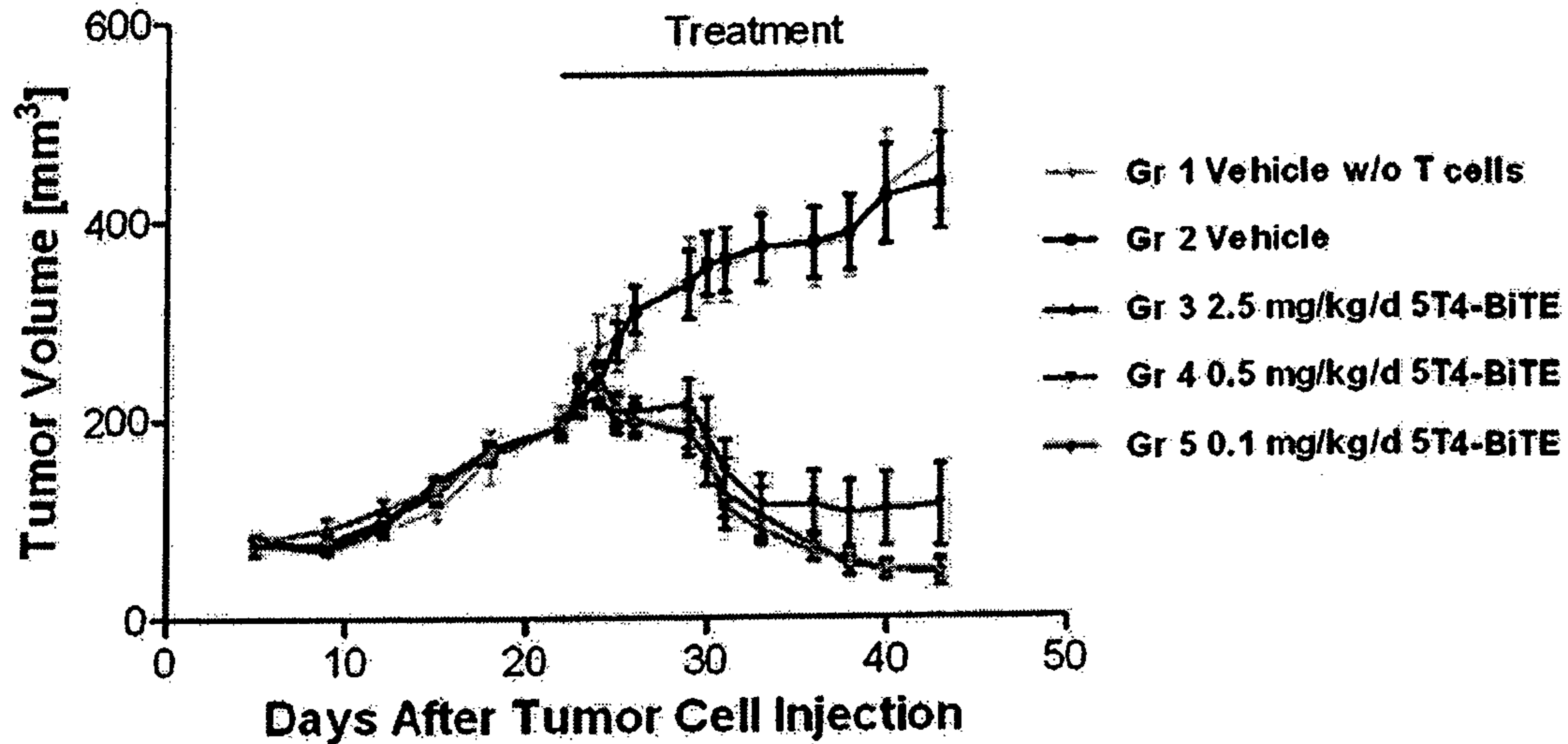


Figure 2

