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(54) Titre : ANTICORPS MONOCLONAUX CONTRE HER2  
(54) Title: MONOCLONAL ANTIBODIES AGAINST HER2

IgHV4-34-01 / IGHJ4-02 – VH alignment (Group 2, No. 1)

|             |                                                                                                                                                                                                                                                         |
|-------------|---------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| IgHV4-34-01 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR                                                                                                                                                                                      |
| TH1014-025  | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR                                                                                                                                                                                      |
| VH1014-001  | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWNWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR                                                                                                                                                                                      |
| VH1014-143  | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWNWIRQPPGKGLEWIGEINHSGSANYNPSLMSR                                                                                                                                                                                      |
| VH1014-019  | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEINHVGSTNYNPSLKSR                                                                                                                                                                                      |
| VH1014-021  | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR                                                                                                                                                                                      |
| VH1014-027  | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYFWNWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR                                                                                                                                                                                      |
| Consensus   | QVQLQQWGAGLLKPSETLSLTCAVYGGSF <del>S</del> <del>X</del> <del>Y</del> <del>X</del> <del>X</del> WIRQPPGKGLEWIGE <del>I</del> <del>X</del> <del>H</del> <del>X</del> <del>G</del> <del>S</del> <del>X</del> NYNPSL <del>X</del> <del>S</del> <del>R</del> |
| IgHV4-34-01 | VTISVDTSKNQFSLKLSSVTAADTAVYYCAR-----YFDYWGGQGLVTVSS                                                                                                                                                                                                     |
| TH1014-025  | VTISVDTSKNQFSLKLSSVTAADTAVYYCARGYYDSGVYYFDYWAQGLVTVSS                                                                                                                                                                                                   |
| VH1014-001  | VTISVDTSKNQFSLKLSSVTAADTAVYYCARGNYGSGYYYFDLWGRGTQVTVSS                                                                                                                                                                                                  |
| VH1014-143  | VTISVDTSKNQFSLQLSSVTAADTAVYYCARGYYGSGYYYFDYWGQGLVTVSS                                                                                                                                                                                                   |
| VH1014-019  | VTISVDTSK <del>S</del> QFSLKLSSVTAADTAVYYCARGYYDSGVYYFDYWAQGLVTVSS                                                                                                                                                                                      |
| VH1014-021  | VTISVDTSKNQFSLKLSSVTAADTAVYYCARGYYASGVYYFDYWGQGLVTVSS                                                                                                                                                                                                   |
| VH1014-027  | VTISVDTSKNQFSLNLSSVTAADTAVYYCARGLLGSGYYYFDYWDQGLVTVSS                                                                                                                                                                                                   |
| Consensus   | VTISVDTSK <del>X</del> QFSL <del>X</del> LSSVTAADTAVYYCARG <del>X</del> <del>X</del> <del>X</del> SG <del>X</del> <del>X</del> <del>X</del> YFD <del>X</del> <del>X</del> <del>X</del> GTXVTVSS                                                         |

(57) **Abrégé/Abstract:**  
This invention relates to a monoclonal antibody which binds human epidermal growth factor receptor 2 (HER2) and which comprises a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOS:134, 135, and 136, respectively, and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:27, AAS, and SEQ ID NO:28 respectively.



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### **Abstract**

This invention relates to a monoclonal antibody which binds human epidermal growth factor receptor 2 (HER2) and which comprises a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOS:134, 135, and 136, respectively, and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:27, AAS, and SEQ ID NO:28 respectively.

## MONOCLONAL ANTIBODIES AGAINST HER2

This application is a divisional application of co-pending application Serial No. 2,800,785 filed November 26, 2012.

### FIELD OF THE INVENTION

The present invention relates to monoclonal antibodies directed to human epidermal growth factor receptor 2 (HER2) and to uses of such antibodies, in particular their use in the treatment of cancer.

### BACKGROUND OF THE INVENTION

HER2 is a 185-kDa cell surface receptor tyrosine kinase and member of the epidermal growth factor receptor (EGFR) family that comprises four distinct receptors: EGFR/ErbB-1, HER2/ErbB-2, HER3/ErbB-3, and HER4/ErbB-4. Both homo- and heterodimers are formed by the four members of the EGFR family, with HER2 being the preferred and most potent dimerization partner for other ErbB receptors (Graus-Porta *et al.*, *Embo J* 1997;16:1647-1655; Tao *et al.*, *J Cell Sci* 2008;121:3207-3217). HER2 can be activated by overexpression or by heterodimerization with other ErbBs that can be activated by ligand binding (Riese and Stern, *Bioessays* 1998;20:41-48). For HER2, no ligand has been identified. HER2 activation leads to receptor phosphorylation, which triggers a cascade of downstream signals through multiple signaling pathways, such as MAPK, phosphoinositol 3-kinase/AKT, JAK/STAT and PKC, which ultimately results in the regulation of multiple cellular functions, such as growth, survival and differentiation (Huang *et al.*, *Expert Opin Biol Ther* 2009;9:97-110).

Much of the attention on HER2 in tumors has been focused on its role in breast cancer, in which HER2 overexpression is reported in approximately 20% of the cases and is correlated with poor prognosis (Reese *et al.*, *Stem Cells* 1997;15:1-8; Andrechek *et al.*, *Proc Natl Acad Sci U S A* 2000;97:3444-3449; and Slamon *et al.*, *Science* 1987;235:177-182). Besides breast cancer, HER2 expression has also been associated with other human carcinoma types, including prostate cancer, non-small cell lung cancer, bladder cancer, ovarian cancer, gastric cancer, colon cancer, esophageal cancer and squamous cell carcinoma of the head & neck (Garcia de Palazzo *et al.*, *Int J Biol Markers* 1993;8:233-239; Ross *et al.*, *Oncologist* 2003;8:307-325; Osman *et al.*, *J Urol* 2005;174:2174-2177; Kapitanovic *et al.*, *Gastroenterology* 1997;112:1103-1113; Turken *et al.*, *Neoplasma* 2003;50:257-261; and Oshima *et al.*, *Int J Biol Markers* 2001;16:250-254).

Trastuzumab (Herceptin®) is a recombinant, humanized monoclonal antibody directed against domain IV of the HER2 protein, thereby blocking ligand-independent HER2 homodimerization, and to a lesser extent heterodimerization of HER2 with other family members in cells with high HER2 overexpression (Cho *et al.*, *Nature* 2003;421:756-760 and

Wehrman *et al.*, Proc Natl Acad Sci U S A 2006;103:19063-19068). In cells with modest HER2 expressing levels, trastuzumab was found to inhibit the formation of HER2/EGFR heterodimers (Wehrman *et al.*, (2006), *supra*; Schmitz *et al.*, Exp Cell Res 2009;315:659-670). Trastuzumab mediates antibody-dependent cellular cytotoxicity (ADCC) and prevents ectodomain shedding, which would otherwise result in the formation of a truncated constitutively active protein in HER2 overexpressing cells. Also inhibition of both *in vitro* and *in vivo* proliferation of tumor cells expressing high levels of HER2 has been reported for trastuzumab (reviewed in Nahta and Esteva, Oncogene 2007;26:3637-3643). Herceptin® has been approved both for first-line and adjuvant treatment of HER2 overexpressing metastatic breast cancer, either in combination with chemotherapy, or as a single agent following one or more chemotherapy regimens. Trastuzumab has been found to be effective only in 20-50% of HER2 overexpressing breast tumor patients and many of the initial responders show relapse after a few months (Dinh *et al.*, Clin Adv Hematol Oncol 2007;5:707-717).

Pertuzumab (Omnitarg™) is another humanized monoclonal antibody. It is directed against domain II of the HER2 protein, resulting in inhibition of ligand-induced heterodimerization (i.e., HER2 dimerizing with another member of the ErbB family to which a ligand has bound); a mechanism reported to not strictly require high HER2 expression levels (Franklin *et al.*, Cancer Cell 2004;5:317-328.). Although pertuzumab also mediates ADCC, the main mechanism of action of pertuzumab relies on its dimerization blockade (Hughes *et al.*, Mol Cancer Ther 2009;8:1885-1892). Moreover, pertuzumab was found to enhance EGFR internalization and downregulation by inhibiting the formation of EGFR/HER2 heterodimers, which otherwise tethers EGFR at the plasma membrane (Hughes *et al.*, 2009, *supra*). This correlates with the observation that EGFR homodimers internalize more efficient than EGFR/HER2 dimers (Pedersen *et al.*, Mol Cancer Res 2009;7:275-284. The complementary mechanisms of action of pertuzumab and trastuzumab reportedly results in enhanced anti-tumor effects and efficacy when combined in patients who progressed during prior trastuzumab therapy (Baselga *et al.*, J Clin Oncol 2010;28:1138-1144), and a phase III trial to evaluate this antibody combination together with Docetaxel in previously untreated HER2-positive metastatic breast cancer is underway.

An alternative approach to improve targeted antibody therapy is by delivering cytotoxic cells or drugs specifically to the antigen-expressing cancer cells. For example, the so-called trifunctional antibodies are bispecific antibodies, targeting with one arm the antigen on the tumor cell and with the other arm for instance CD3 on T cells. Upon binding, a complex of T cells, tumor cells and effector cells that bind Fc is formed, leading to killing of the tumor cells (Muller and Kontermann, BioDrugs 2010;24:89-98.). Ertumaxomab is one

such trifunctional antibody against HER2, which induces cytotoxicity in cell lines with low HER2 expression and which is in Phase II clinical development in metastatic breast cancer (Jones *et al.*, Lancet Oncol 2009;10:1179-1187 and Kiewe *et al.*, Clin Cancer Res 2006;12:3085-3091).

A HER2 antibody drug conjugate (ADC) is currently in clinical development. T-DM1 consists of trastuzumab conjugated to the fungal toxin maytansine. In Phase II trials, responses in a heavily pretreated patient cohort including prior trastuzumab and/or lapatinib therapy were reported Burris *et al.*, 2011, J Clin Oncol 29: 398-405 and Lewis Phillips *et al.*, Cancer Res 2008;68:9280-9290). Preliminary data from a Phase II trial determining efficacy and safety of T-DM1 versus trastuzumab plus docetaxel in her2-positive metastatic breast cancer patients with no prior chemotherapy for metastatic disease were reported (Perez *et al.*, Abstract BA3, European Society for Medical Oncology meeting 2010). A Phase III trial to evaluate T-DM1 efficacy and safety versus capecitabine + lapatinib in patients with HER2-positive locally advanced or metastatic breast cancer who received prior trastuzumab therapy is ongoing.

While many factors are involved in selecting a suitable antibody for HER2 targeted therapy, it is typically an advantage for an ADC approach if the HER2-antibody complex efficiently internalizes upon antibody binding. Studies on murine HER2 antibodies have shown that certain combinations of antibodies instigate HER2 endocytosis (Ben-Kasus *et al.*, PNAS 2009;106:3294-9). Human HER2 antibodies F5 and C1 have been reported to internalize relatively rapidly on their own and to bind the same epitope (WO 99/55367 and WO 2006/116107). As compared to EGFR, however, internalization of HER2 is impaired. Indeed, EGFR homodimers internalize much more efficiently than HER2 homodimers (Dinh *et al.*, Clin Adv Hematol Oncol 2007;5:707-717). EGFR, and also HER3, can increase endocytosis of HER2 by the formation of EGFR/HER2 and HER3/HER2 heterodimers, respectively (Baulida *et al.*, J Biol Chem 1996;271:5251-5257; Pedersen NM, *et al.*, Mol Cancer Res 2009;7:275-84).

The complex mechanisms regulating the function of HER2 warrant further research on new and optimized therapeutic strategies against this proto-oncogene. Accordingly, there remains a need for effective and safe products for treating HER2-related diseases, such as cancer.

#### **SUMMARY OF THE INVENTION**

It is an object of the present invention to provide novel highly specific and effective monoclonal HER2 antibodies for medical use. The antibodies of the invention exhibit HER2 binding characteristics that differ from antibodies described in the art. Particularly, although most of the antibodies apparently bind to HER2 segments overlapping with those bound by

trastuzumab, pertuzumab or F5/C1 as shown in a cross-blocking HER2 binding assay, the novel antibodies are characterized by a higher efficiency in killing HER2-expressing tumor cells in an ADC assay, improved internalization and/or other advantages over the known antibodies.

In preferred embodiments, the antibodies of the invention are fully human, bind to novel epitopes, and/or have favorable properties for therapeutic use in human patients. Exemplary properties include, but are not limited to, favorable binding characteristics to cancer cells expressing human HER2 at high or low levels, specific binding rhesus epithelial cells expressing a HER2 ortholog, efficient internalization upon binding to HER2, high capacity for killing cancer cells expressing high- or low-levels of HER2 when administered as an ADC, promoting proliferation of HER2-expressing cells less than F5, a neutral or inhibitory effect on the proliferation of HER2-expressing cancer cells, and provide for effective ADCC-mediated killing of HER2-expressing cells, as well as any combination of the foregoing properties.

These and other aspects of the invention are described in further detail below.

#### **BRIEF DESCRIPTION OF THE DRAWINGS**

**Figure 1:** Alignment of HuMab heavy chain variable region (VH) sequences with germline (reference) sequences (A-K). In each VH sequence, the amino acids that differ from those of the germline (reference) at specific positions are highlighted. Consensus VH sequences are shown, where "X" indicates positions at which alternative amino acids (selected from those aligned at each position) are possible. The CDR1, CDR2, and CDR3 sequences are underlined in each VH sequence. The consensus CDR sequences are further defined in Table 4.

**Figure 2:** Alignment of HuMab light chain variable region (VL) sequences with germline (reference) sequences (panels A-B). In each VL sequence, the amino acids that differ from those of the germline (reference) at specific positions are highlighted. In Figure 2A, all VL sequences derived from the same V-segment (IgKV1-12-01), but the closest J-segment differed between antibodies. Consensus VL sequences are shown, where "X" indicates positions at which alternative amino acids (selected from those aligned at the indicated position) are possible. The CDR1, CDR2, and CDR3 sequences are underlined in each VL sequence. The consensus CDR sequences are further defined in Table 4.

**Figure 3:** Binding curves of HER2 antibodies to (A, B) high (AU565) and (C, D) low (A431) HER2 expressing cell lines, determined as described in Example 12. Data shown are mean fluorescence intensities (MFI) of one representative experiment for each cell line. The EC<sub>50</sub> values indicate the apparent affinities.

**Figure 4:** Binding of HER2 antibodies to HER2 expressed on monkey Rhesus epithelial cells. Data shown are mean fluorescence intensities (MFI) of one experiment, described in Example 13.

**Figure 5:** Chromium-release (ADCC) assay of HER2 antibodies, showing PBMC-mediated lysis of <sup>51</sup>Cr-labeled SK-BR-3 cells after incubation with HER2 antibody. Values depicted are the mean maximum percentages <sup>51</sup>Cr-release ± the standard deviation from one representative *in vitro* ADCC experiment with SK-BR-3 cells. See Example 15 for details.

**Figure 6:** Effect of HER2 antibodies on the proliferation of AU565 cells, as compared to untreated cells (set to 100%). Data shown are percentages proliferation of AU565 cells compared to untreated cells measured in three independent experiments ± the standard deviation. \* Significant (P<0.05). See Example 16 for details.

**Figure 7:** Percentage of viable MCF7 cells stimulated with Heregulin-β1 and treated with the indicated HER2 antibodies, relative to cells stimulated with Heregulin-β1 only. As a control, the percentage proliferation of unstimulated cells is shown (none). Data was obtained from three independent experiments ± the stdev. \* Significant inhibition of Heregulin-β1-induced proliferation (P<0.05). See Example 17 for details.

**Figure 8:** ADC assay, showing killing of AU565 cells (A, B) or A431 cells (C, D) via anti-kappa-ETA'-conjugated HER2 antibodies. (A, B) Data shown are fluorescence intensities (FI) of one representative experiment with AU565 cells treated with non-conjugated and anti-kappa-ETA'-conjugated HER2 antibodies. (C, D) Data shown are mean fluorescence intensities (MFI) of one representative experiment with A431 cells treated with non-conjugated and anti-kappa-ETA'-conjugated HER2 antibodies. See Example 18 for details.

**Figure 9:** Killing of A431 cells induced by anti-kappa-ETA' pre-incubated HER2 x HER2 bispecific antibodies. The viability of A431 cells after 3 days incubation with HER2 antibodies, pre-incubated with anti-kappa-ETA'. Cell viability was quantified using Alamarblue. Data shown are fluorescence intensities (FI) of one experiment with A431 cells treated with anti-kappa-ETA'-conjugated HER2 antibodies and HER2 x HER2 bispecific antibodies. Staurosporin was used as positive control, whereas an isotype control antibody was used as negative control.

**Figure 10:** HER2 x HER2 bispecific molecules induced downmodulation of HER2 receptor. Relative percentage of HER2 expression levels in AU565 cell lysates after 3 days incubation with 10 µg/mL mAb. The amount of HER2 was quantified using a HER2-specific capture ELISA and depicted as percentage inhibition compared to untreated cells. Data shown is the mean of two experiments plus standard deviation, except for combinations of monospecific IgG1 antibodies which were tested once.

**Figure 11:** Colocalization analysis of HER2 x HER2 bispecific antibodies (FITC) with lysosomal marker LAMP1 (Cy5). FITC pixel intensity overlapping with Cy5 for various monospecific HER2 antibodies and HER2 x HER2 bispecific antibodies (Figure 11(A)) FITC pixel intensity in LAMP1/Cy5 positive pixels of three different images is plotted for each antibody tested. Monospecifics show lower FITC pixel intensities in the LAMP1/Cy5 positive pixels compared to bispecifics. Figure 11(B) represents the mean value of FITC pixel intensity per LAMP1/Cy5 positive pixel calculated from the three different images. Together these results indicate that after internalization higher levels of bispecific antibodies, compared to monospecifics antibodies, localize to Lamp1/Cy5 positive vesicles.

**Figure 12:** Inhibition of proliferation by HER-2 mono and bispecific antibodies. AU565 cells were seeded in the presence of 10 µg/mL HER2 antibody or HER2 x HER2 bispecific antibody in serum-free cell culture medium. After three days, the amount of viable cells was quantified with Alamarblue and cell viability was presented as a percentage relative to untreated cells. An isotype control antibody was used as negative control. Data shown are percentage viable AU565 cells compared to untreated cells measured in five-fold  $\pm$  the standard deviation. \* indicates only one data point was depicted.

**Figure 13:** T cell mediated cytotoxicity of AU565 cells by HER2 x CD3 bispecific antibodies as well as by N297Q mutants of HER2 x CD3 bispecific antibodies (bispecific indicated as Duo in the figure).

**Figure 14:** Antibody induced downmodulation of HER2. Relative percentage of HER2 expressed in AU565 cell lysate after 3 days incubation with 10 µg/mL antibody. The amount of HER2 was quantified using a HER2-specific capture ELISA and plotted as a percentage relative to untreated cells. Data shown are mean of three experiments  $\pm$  standard deviation.

**Figure 15:** Colocalization analysis of HER2 antibodies (FITC) with lysosomal marker LAMP1 (Cy5). FITC pixel intensity overlapping with Cy5 for various monospecific HER2 antibodies. FITC pixel intensity in LAMP1/Cy5 positive pixels of three different images is plotted for each antibody. Group 3 antibodies 098 and 153 show higher FITC pixel intensities in the LAMP1/Cy5 positive compartments compared to antibodies 025 and pertuzumab from Group 2 and 169 and Herceptin from Group 1.

**Figure 16:** HER2 antibody binding to CHO-S cells transfected with different HER2 ECD construct analyzed by means of flow cytometry. Hu-HER2 = fully human HER2, Hu-HER2-ch(I) CR1 = hu-HER2 with chicken domain I, Hu-HER2-ch(II) = hu-HER2 with chicken domain II, hu-HER2-ch(III) = hu-HER2 with chicken domain III and Hu-HER2-ch(IV) = hu-HER2 with chicken domain IV. Data shown are mean fluorescence intensities (MFI) of one representative antibody, TH1014-153. See example 25 for details.

**Figure 17:** In vivo effect of HER2-HuMabs in the NCI-N87 human gastric carcinoma xenograft model in female CB.17 severe combined immunodeficiency (SCID) mice. Data shown are mean tumorsize  $\pm$  S.E.M. per group (n = 10 mice per group) (A) and survival (B). See example 29 for details.

**Figure 18:** In vivo effect of HER2 HuMabs in BT-474 breast tumor xenografts in Balb/C nude mice. Data shown are mean tumorsize  $\pm$  S.E.M. per group (n = 8 mice per group) (A) and survival (B). See example 30 for details.

## **DETAILED DESCRIPTION OF THE INVENTION**

### Definitions

The term "HER2" (also known as ErbB-2, NEU, HER-2, and CD340), when used herein, refers to human epidermal growth factor receptor 2 (SwissProt P04626) and includes any variants, isoforms and species homologs of HER2 which are naturally expressed by cells, including tumor cells, or are expressed on cells transfected with the HER2 gene. Species homologs include rhesus monkey HER2 (macaca mulatta; Genbank accession No. GI:109114897).

The term "immunoglobulin" refers to a class of structurally related glycoproteins consisting of two pairs of polypeptide chains, one pair of light (L) low molecular weight chains and one pair of heavy (H) chains, all four inter-connected by disulfide bonds. The structure of Immunoglobulins has been well characterized. See for instance Fundamental Immunology Ch. 7 (Paul, W., ed., 2nd ed. Raven Press, N.Y. (1989)). Briefly, each heavy chain typically is comprised of a heavy chain variable region (abbreviated herein as  $V_H$  or  $VH$ ) and a heavy chain constant region. The heavy chain constant region typically is comprised of three domains,  $C_{H1}$ ,  $C_{H2}$ , and  $C_{H3}$ . Each light chain typically is comprised of a light chain variable region (abbreviated herein as  $V_L$  or  $VL$ ) and a light chain constant region. The light chain constant region typically is comprised of one domain,  $C_L$ . The  $V_H$  and  $V_L$  regions may be further subdivided into regions of hypervariability (or hypervariable regions which may be hypervariable in sequence and/or form of structurally defined loops), also termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FRs). Each  $V_H$  and  $V_L$  is typically composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4 (see also Chothia and Lesk J. Mol. Biol. 196, 901-917 (1987)). Unless otherwise stated or contradicted by context, CDR sequences herein are identified according to IMGT rules (Brochet X., Nucl Acids Res. 2008;36:W503-508 and Lefranc MP., Nucleic Acids Research 1999;27:209-212. However, the numbering of amino acid residues in an antibody sequence can also be

performed by the method described in Kabat *et al.*, Sequences of Proteins of Immunological Interest, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991) (phrases such as "variable domain residue numbering as in Kabat", "Kabat position" or "according to Kabat" herein refer to this numbering system). Particularly, for numbering of amino acids in the constant region, the EU index numbering system according to Kabat *et al.*, *supra*, can be used. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

The term "antibody" (Ab) in the context of the present invention refers to an immunoglobulin molecule, a fragment of an immunoglobulin molecule, or a derivative of either thereof, which has the ability to specifically bind to an antigen under typical physiological conditions with a half life of significant periods of time, such as at least about 30 minutes, at least about 45 minutes, at least about one hour, at least about two hours, at least about four hours, at least about 8 hours, at least about 12 hours, about 24 hours or more, about 48 hours or more, about 3, 4, 5, 6, 7 or more days, etc., or any other relevant functionally-defined period (such as a time sufficient to induce, promote, enhance, and/or modulate a physiological response associated with antibody binding to the antigen and/or time sufficient for the antibody to recruit an effector activity). The variable regions of the heavy and light chains of the immunoglobulin molecule contain a binding domain that interacts with an antigen. The constant regions of the antibodies (Abs) may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (such as effector cells) and components of the complement system such as C1q, the first component in the classical pathway of complement activation. A HER2 antibody may also be a bispecific antibody, diabody, or similar molecule (see for instance PNAS USA 90(14), 6444-8 (1993) for a description of diabodies). Indeed, bispecific antibodies, diabodies, and the like, provided by the present invention may bind any suitable target in addition to a portion of HER2. As indicated above, the term antibody herein, unless otherwise stated or clearly contradicted by context, includes fragments of an antibody that are antigen-binding fragments, i.e., retain the ability to specifically bind to the antigen. It has been shown that the antigen-binding function of an antibody may be performed by fragments of a full-length antibody. Examples of antigen-binding fragments encompassed within the term "antibody" include (i) a Fab' or Fab fragment, a monovalent fragment consisting of the V<sub>L</sub>, V<sub>H</sub>, C<sub>L</sub> and C<sub>H</sub>1 domains, or a monovalent antibody as described in WO2007059782 (Genmab); (ii) F(ab')<sub>2</sub> fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting essentially of the V<sub>H</sub> and C<sub>H</sub>1 domains; (iv) a Fv fragment consisting essentially of the V<sub>L</sub>

and V<sub>H</sub> domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, Nature 341, 544-546 (1989)), which consists essentially of a V<sub>H</sub> domain and also called domain antibodies (Holt *et al.*; Trends Biotechnol. 2003 Nov;21(11):484-90); (vi) camelid or nanobodies (Revetz *et al.*; Expert Opin Biol Ther. 2005 Jan;5(1):111-24) and (vii) an isolated complementarity determining region (CDR). Furthermore, although the two domains of the Fv fragment, V<sub>L</sub> and V<sub>H</sub>, are coded for by separate genes, they may be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V<sub>L</sub> and V<sub>H</sub> regions pair to form monovalent molecules (known as single chain antibodies or single chain Fv (scFv), see for instance Bird *et al.*, Science 242, 423-426 (1988) and Huston *et al.*, PNAS USA 85, 5879-5883 (1988)). Such single chain antibodies are encompassed within the term antibody unless otherwise noted or clearly indicated by context. Although such fragments are generally included within the meaning of antibody, they collectively and each independently are unique features of the present invention, exhibiting different biological properties and utility. These and other useful antibody fragments in the context of the present invention, as well as bispecific formats of such fragments, are discussed further herein. It also should be understood that the term antibody, unless specified otherwise, also includes polyclonal antibodies, monoclonal antibodies (mAbs), antibody-like polypeptides, such as chimeric antibodies and humanized antibodies, and antibody fragments retaining the ability to specifically bind to the antigen (antigen-binding fragments) provided by any known technique, such as enzymatic cleavage, peptide synthesis, and recombinant techniques. An antibody as generated can possess any isotype.

As used herein, "isotype" refers to the immunoglobulin class (for instance IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM) that is encoded by heavy chain constant region genes.

The term "monovalent antibody" means in the context of the present invention that an antibody molecule is capable of binding a single molecule of the antigen, and thus is not able of antigen crosslinking.

An "antibody deficient in effector function" or an "effector-function-deficient antibody" refers to an antibody which has a significantly reduced or no ability to activate one or more effector mechanisms, such as complement activation or Fc receptor binding. Thus, effector-function deficient antibodies have significantly reduced or no ability to mediate antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC). An example of such an antibody is IgG4.

A "HER2 antibody" or "anti-HER2 antibody" is an antibody as described above, which binds specifically to the antigen HER2.

The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. 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 mutagenesis *in vitro* or by somatic mutation *in vivo*). However, the term "human antibody", as used herein, is not intended to include antibodies in which CDR sequences derived from the germline of another mammalian species, such as a mouse, have been grafted onto human framework sequences.

As used herein, a human antibody is "derived from" a particular germline sequence if the antibody is obtained from a system using human immunoglobulin sequences, for instance by immunizing a transgenic mouse carrying human immunoglobulin genes or by screening a human immunoglobulin gene library, and wherein the selected human antibody is at least 90%, such as at least 95%, for instance at least 96%, such as at least 97%, for instance at least 98%, or such as at least 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, outside the heavy chain CDR3, a human antibody derived from a particular human germline sequence will display no more than 20 amino acid differences, *e.g.* no more than 10 amino acid differences, such as no more than 9, 8, 7, 6 or 5, for instance no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

In a preferred embodiment, the antibody of the invention is isolated. An "isolated antibody," as used herein, is intended to refer to an antibody which is substantially free of other antibodies having different antigenic specificities (for instance an isolated antibody that specifically binds to HER2 is substantially free of antibodies that specifically bind antigens other than HER2). An isolated antibody that specifically binds to an epitope, isoform or variant of HER2 may, however, have cross-reactivity to other related antigens, for instance from other species (such as HER2 species homologs). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals. In one embodiment of the present invention, two or more "isolated" monoclonal antibodies having different antigen-binding specificities are combined in a well-defined composition.

When used herein in the context of two or more antibodies, the term "competes with" or "cross-competes with" indicates that the two or more antibodies compete for binding to HER2, *e.g.* compete for HER2 binding in the assay described in Example 14. An antibody "blocks" or "cross-blocks" one or more other antibodies from binding to HER2 if the antibody competes with the one or more other antibodies 25% or more, with 25%-74% representing "partial block" and 75%-100% representing "full block", preferably as

determined using the assay of Example 14. For some pairs of antibodies, competition or blocking in the assay of the Examples is only observed when one antibody is coated on the plate and the other is used to compete, and not vice versa. Unless otherwise defined or negated by context, the terms "competes with", "cross-competes with", "blocks" or "cross-blocks" when used herein is also intended to cover such pairs of antibodies.

The term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and nonconformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. The epitope may comprise amino acid residues directly involved in the binding (also called immunodominant component of the epitope) and other amino acid residues, which are not directly involved in the binding, such as amino acid residues which are effectively blocked or covered by the specifically antigen binding peptide (In other words, the amino acid residue is within the footprint of the specifically antigen binding peptide).

The term "monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Accordingly, the term "human monoclonal antibody" refers to antibodies displaying a single binding specificity which have variable and constant regions derived from human germline immunoglobulin sequences. The human monoclonal antibodies may be generated by a hybridoma which includes a B cell obtained from a transgenic or transchromosomal nonhuman animal, such as a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene, fused to an immortalized cell.

As used herein, the term "binding" in the context of the binding of an antibody to a predetermined antigen or epitope typically is a binding with an affinity corresponding to a  $K_D$  of about  $10^{-7}$  M or less, such as about  $10^{-8}$  M or less, such as about  $10^{-9}$  M or less, about  $10^{-10}$  M or less, or about  $10^{-11}$  M or even less when determined by for instance surface plasmon resonance (SPR) technology in a BIAcore™ 3000 instrument using the antigen as the ligand and the antibody as the analyte, and binds to the predetermined antigen with an affinity corresponding to a  $K_D$  that is at least ten-fold lower, such as at least 100 fold lower, for instance at least 1,000 fold lower, such as at least 10,000 fold lower, for instance at least 100,000 fold lower than its affinity for binding to a non-specific antigen (e.g., BSA, casein) other than the predetermined antigen or a closely-related antigen. The amount with which the affinity is lower is dependent on the  $K_D$  of the antibody, so that when the  $K_D$  of

the antibody is very low (that is, the antibody is highly specific), then the amount with which the affinity for the antigen is lower than the affinity for a non-specific antigen may be at least 10,000 fold.

The term " $k_d$ " ( $\text{sec}^{-1}$ ), as used herein, refers to the dissociation rate constant of a particular antibody-antigen interaction. Said value is also referred to as the  $k_{\text{off}}$  value.

The term " $k_a$ " ( $\text{M}^{-1} \times \text{sec}^{-1}$ ), as used herein, refers to the association rate constant of a particular antibody-antigen interaction.

The term " $K_D$ " (M), as used herein, refers to the dissociation equilibrium constant of a particular antibody-antigen interaction.

The term " $K_A$ " ( $\text{M}^{-1}$ ), as used herein, refers to the association equilibrium constant of a particular antibody-antigen interaction and is obtained by dividing the  $k_a$  by the  $k_d$ .

As used herein, the term "inhibits proliferation" (e.g. referring to cells, such as tumor cells) is intended to include any substantial decrease in the cell proliferation when contacted with a HER2 antibody as compared to the proliferation of the same cells not in contact with a HER2 antibody, e.g., the inhibition of proliferation of a cell culture by at least about 10%, at least about 20% or at least about 30%, or at least as much as a reference antibody such as trastuzumab, e.g., as determined by an assay in the Examples, e.g. example 16.

As used herein, the term "promotes proliferation" (e.g. referring to cells, such as tumor cells) is intended to include any substantial increase in the cell proliferation when contacted with a HER2 antibody as compared to the proliferation of the same cells not in contact with a HER2 antibody, e.g., the promotion of proliferation of a cell culture by at least about 10%, at least about 20% or at least about 30%, or at least as much as a reference antibody as F5, e.g., as determined by an assay in the Examples.

As used herein, the term "internalization", when used in the context of a HER2 antibody includes any mechanism by which the antibody is internalized into a HER2-expressing cell from the cell-surface and/or from surrounding medium, e.g., via endocytosis. The internalization of an antibody can be evaluated using a direct assay measuring the amount of internalized antibody (such as, e.g., the fab-CypHer5E assay described in Example 18), or an indirect assay where the effect of an internalized antibody-toxin conjugate is measured (such as, e.g., the anti-kappa-ETA' assay of Example 17).

The present invention also provides antibodies comprising functional variants of the  $V_L$  region,  $V_H$  region, or one or more CDRs of the antibodies of the examples. A functional variant of a  $V_L$ ,  $V_H$ , or CDR used in the context of a HER2 antibody still allows the antibody to retain at least a substantial proportion (at least about 50%, 60%, 70%, 80%, 90%, 95% or more) of the affinity/avidity and/or the specificity/selectivity of the parent antibody and

in some cases such a HER2 antibody may be associated with greater affinity, selectivity and/or specificity than the parent antibody.

Such functional variants typically retain significant sequence identity to the parent antibody. The percent identity between two sequences is a function of the number of identical positions shared by the sequences (i.e., % homology = # of identical positions/total # of positions x 100), taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. The percent identity between two nucleotide or amino acid sequences may e.g. be determined using the algorithm of E. Meyers and W. Miller, *Comput. Appl. Biosci* 4, 11-17 (1988) which has been incorporated into the ALIGN program (version 2.0), using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. In addition, the percent identity between two amino acid sequences may be determined using the Needleman and Wunsch, *J. Mol. Biol.* 48, 444-453 (1970) algorithm.

Exemplary variants include those which differ from a parent antibody VH and/or VL sequence shown in Figures 1 and 2 at one or more "variant" amino acid positions, denoted "X" in the corresponding consensus sequence. Preferred variants are those in which the new amino acid is selected from those at the corresponding position in one of the aligned sequences in Figure 1 or 2 (for details on CDR sequence variants, see Table 4). Alternatively or additionally, the sequence of VH, VL or CDR variants may differ from the sequence of the VH, VL or CDR of the parent antibody sequences mainly by conservative substitutions; for instance at least 10, such as at least 9, 8, 7, 6, 5, 4, 3, 2 or 1 of the substitutions in the variant are conservative amino acid residue replacements.

In the context of the present invention, conservative substitutions may be defined by substitutions within the classes of amino acids reflected in the following table:

**Amino acid residue classes for conservative substitutions**

|                                |                                                 |
|--------------------------------|-------------------------------------------------|
| Acidic Residues                | Asp (D) and Glu (E)                             |
| Basic Residues                 | Lys (K), Arg (R), and His (H)                   |
| Hydrophilic Uncharged Residues | Ser (S), Thr (T), Asn (N), and Gln (Q)          |
| Aliphatic Uncharged Residues   | Gly (G), Ala (A), Val (V), Leu (L), and Ile (I) |
| Non-polar Uncharged Residues   | Cys (C), Met (M), and Pro (P)                   |
| Aromatic Residues              | Phe (F), Tyr (Y), and Trp (W)                   |

The term "recombinant host cell" (or simply "host cell"), as used herein, is intended to refer to a cell into which an expression vector has been introduced, e.g. an expression vector encoding an antibody of the invention. Recombinant host cells include, for example, transfectomas, such as CHO cells, HEK293 cells, NS/0 cells, and lymphocytic cells.

The term "transgenic non-human animal" refers to a non-human animal having a genome comprising one or more human heavy and/or light chain transgenes or transchromosomes (either integrated or non-integrated into the animal's natural genomic DNA) and which is capable of expressing fully human antibodies. For example, a transgenic mouse can have a human light chain transgene and either a human heavy chain transgene or human heavy chain transchromosome, such that the mouse produces human HER2 antibodies when immunized with HER2 antigen and/or cells expressing HER2. The human heavy chain transgene may be integrated into the chromosomal DNA of the mouse, as is the case for transgenic mice, for instance HuMAb mice, such as HCo7, HCo12, or HCo17 mice, or the human heavy chain transgene may be maintained extrachromosomally, as is the case for transchromosomal KM mice as described in WO02/43478. Similar mice, having a larger human Ab gene repertoire, include HCo7 and HCo20 (see e.g. WO2009097006). Such transgenic and transchromosomal mice (collectively referred to herein as "transgenic mice") are capable of producing multiple isotypes of human monoclonal antibodies to a given antigen (such as IgG, IgA, IgM, IgD and/or IgE) by undergoing V-D-J recombination and isotype switching. Transgenic, nonhuman animal can also be used for production of antibodies against a specific antigen by introducing genes encoding such specific antibody, for example by operatively linking the genes to a gene which is expressed in the milk of the animal.

"Treatment" refers to the administration of an effective amount of a therapeutically active compound of the present invention with the purpose of easing, ameliorating, arresting or eradicating (curing) symptoms or disease states.

An "effective amount" or "therapeutically effective amount" refers to an amount effective, at dosages and for periods of time necessary, to achieve a desired therapeutic result. A therapeutically effective amount of a HER2 antibody may vary according to factors such as the disease state, age, sex, and weight of the individual, and the ability of the HER2 antibody to elicit a desired response in the individual. A therapeutically effective amount is also one in which any toxic or detrimental effects of the antibody or antibody portion are outweighed by the therapeutically beneficial effects.

An "anti-idiotypic" antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody.

#### Further aspects and embodiments of the invention

As described above, in a first aspect, the invention relates to a monoclonal antibody which binds HER2.

Monoclonal antibodies of the present invention may be produced, e.g., by the hybridoma method first described by Kohler *et al.*, Nature 256, 495 (1975), or may be produced by recombinant DNA methods. Monoclonal antibodies may also be isolated from phage antibody libraries using the techniques described in, for example, Clackson *et al.*, Nature 352, 624-628 (1991) and Marks *et al.*, J. Mol. Biol. 222, 581-597 (1991). Monoclonal antibodies may be obtained from any suitable source. Thus, for example, monoclonal antibodies may be obtained from hybridomas prepared from murine splenic B cells obtained from mice immunized with an antigen of interest, for instance in form of cells expressing the antigen on the surface, or a nucleic acid encoding an antigen of interest. Monoclonal antibodies may also be obtained from hybridomas derived from antibody-expressing cells of immunized humans or non-human mammals such as rats, dogs, primates, etc.

In one embodiment, the antibody of the invention is a human antibody. Human monoclonal antibodies directed against HER2 may be generated using transgenic or transchromosomal mice carrying parts of the human immune system rather than the mouse system. Such transgenic and transchromosomal mice include mice referred to herein as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "transgenic mice".

The HuMAb mouse contains a human immunoglobulin gene miniloci that encodes unrearranged human heavy ( $\mu$  and  $\gamma$ ) and  $\kappa$  light chain immunoglobulin sequences, together with targeted mutations that inactivate the endogenous  $\mu$  and  $\kappa$  chain loci (Lonberg, N. *et al.*, Nature 368, 856-859 (1994)). Accordingly, the mice exhibit reduced expression of mouse IgM or  $\kappa$  and in response to immunization, the introduced human heavy and light chain transgenes, undergo class switching and somatic mutation to generate high affinity human IgG, $\kappa$  monoclonal antibodies (Lonberg, N. *et al.* (1994), *supra*; reviewed in Lonberg, N. Handbook of Experimental Pharmacology 113, 49-101 (1994) , Lonberg, N. and Huszar, D., Intern. Rev. Immunol. Vol. 13 65-93 (1995) and Harding, F. and Lonberg, N. Ann. N.Y. Acad. Sci 764 536-546 (1995)). The preparation of HuMAb mice is described in detail in Taylor, L. *et al.*, Nucleic Acids Research 20, 6287-6295 (1992), Chen, J. *et al.*, International Immunology 5, 647-656 (1993), Tuaillon *et al.*, J. Immunol. 152, 2912-2920 (1994), Taylor, L. *et al.*, International Immunology 6, 579-591 (1994), Fishwild, D. *et al.*, Nature Biotechnology 14, 845-851 (1996). See also US 5,545,806, US 5,569,825, US 5,625,126, US 5,633,425, US 5,789,650, US 5,877,397, US 5,661,016, US 5,814,318, US 5,874,299,

US 5,770,429, US 5,545,807, WO 98/24884, WO 94/25585, WO 93/1227, WO 92/22645, WO 92/03918 and WO 01/09187.

The HCo7, HCo12, HCo17 and HCo20 mice have a JKD disruption in their endogenous light chain (kappa) genes (as described in Chen *et al.*, EMBO J. 12, 821-830 (1993)), a CMD disruption in their endogenous heavy chain genes (as described in Example 1 of WO 01/14424), and a KCo5 human kappa light chain transgene (as described in Fishwild *et al.*, Nature Biotechnology 14, 845-851 (1996)). Additionally, the HCo7 mice have a HCo7 human heavy chain transgene (as described in US 5,770,429), the HCo12 mice have a HCo12 human heavy chain transgene (as described in Example 2 of WO 01/14424), the HCo17 mice have a HCo17 human heavy chain transgene (as described in Example 2 of WO 01/09187) and the HCo20 mice have a HCo20 human heavy chain transgene. The resulting mice express human immunoglobulin heavy and kappa light chain transgenes in a background homozygous for disruption of the endogenous mouse heavy and kappa light chain loci.

In the KM mouse strain, the endogenous mouse kappa light chain gene has been homozygously disrupted as described in Chen *et al.*, EMBO J. 12, 811-820 (1993) and the endogenous mouse heavy chain gene has been homozygously disrupted as described in Example 1 of WO 01/09187. This mouse strain carries a human kappa light chain transgene, KCo5, as described in Fishwild *et al.*, Nature Biotechnology 14, 845-851 (1996). This mouse strain also carries a human heavy chain transchromosome composed of chromosome 14 fragment hCF (SC20) as described in WO 02/43478. HCo12-Balb/C mice can be generated by crossing HCo12 to KCo5[J/K](Balb) as described in WO/2009/097006.

Splenocytes from these transgenic mice may be used to generate hybridomas that secrete human monoclonal antibodies according to well known techniques.

Further, human antibodies of the present invention or antibodies of the present invention from other species may be identified through display-type technologies, including, without limitation, phage display, retroviral display, ribosomal display, and other techniques, using techniques well known in the art and the resulting molecules may be subjected to additional maturation, such as affinity maturation, as such techniques are well known in the art (see for instance Hoogenboom *et al.*, J. Mol. Biol. 227, 381 (1991) (phage display), Vaughan *et al.*, Nature Biotech 14, 309 (1996) (phage display), Hanes and Plucthau, PNAS USA 94, 4937-4942 (1997) (ribosomal display), Parmley and Smith, Gene 73, 305-318 (1988) (phage display), Scott TIBS 17, 241-245 (1992), Cwirla *et al.*, PNAS USA 87, 6378-6382 (1990), Russel *et al.*, Nucl. Acids Research 21, 1081-1085 (1993), Hogenboom *et al.*, Immunol. Reviews 130, 43-68 (1992), Chiswell and McCafferty TIBTECH

10, 80-84 (1992), and US 5,733,743). If display technologies are utilized to produce antibodies that are not human, such antibodies may be humanized.

Antibodies of cross-block group 1

In one aspect of the antibody of the invention, the antibody binds the same epitope on HER2 as one or more of the novel human antibodies of cross-block group 1 described herein.

In one embodiment, the antibody cross-blocks the binding to soluble HER2 of trastuzumab, when determined as described in Example 14.

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5 (**169**).

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:8 and a VL region comprising the sequence of SEQ ID NO:12 (**050**).

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:15 and a VL region comprising the sequence of SEQ ID NO:84 (**084**).

In one embodiment, the antibody binds to the same epitope as a reference antibody comprising VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:77 and a VL region comprising the sequence of SEQ ID NO:78 (**049**);
- b) a VH region comprising the sequence of SEQ ID NO:79 and a VL region comprising the sequence of SEQ ID NO:80 (**051**);
- c) a VH region comprising the sequence of SEQ ID NO:81 and a VL region comprising the sequence of SEQ ID NO:82 (**055**);
- d) a VH region comprising the sequence of SEQ ID NO:83 and a VL region comprising the sequence of SEQ ID NO:84 (**123**);
- e) a VH region comprising the sequence of SEQ ID NO:85 and a VL region comprising the sequence of SEQ ID NO:86 (**161**); and
- f) a VH region comprising the sequence of SEQ ID NO:87 and a VL region comprising the sequence of SEQ ID NO:88 (**124**).

In another additional or alternative aspect of the antibody of the invention, the antibody binds to HER2 and comprises a VH CDR3, VH region and/or VL region sequence similar or identical to a sequence of the novel antibodies described herein.

In one embodiment, the antibody comprises a VH CDR3 region having a sequence selected from the group consisting of

SEQ ID NO:11 (050, 049, 051, 055), optionally wherein the VH region is derived from the IgHV3-21-1 germline sequence;

SEQ ID No:130, such as the sequence of SEQ ID NO:18 (084), optionally wherein the VH region is derived from the IgHV1-69-04 germline sequence;

SEQ ID NO:133 (169, 123, 161, 124), such as the sequence of SEQ ID NO:4 (169), optionally wherein the VH region is derived from the IgHV1-18-1 germline sequence; or

In one embodiment, the antibody comprises a VH CDR3 region of one of antibodies 123, 161, or 124, as shown in Figure 1, optionally wherein the VH region is derived from an IgHV1-18-1 germline.

In one embodiment, the antibody comprises a VH region selected from the group consisting of

- a) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:9, 127 and 11, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID NOS: 9, 10 and 11 (050); optionally where the VH region is derived from an IgHV3-23-1 germline;
- b) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:128, 129 and 130, such the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:16, 17 and 18, respectively (084), optionally where the VH region is derived from an IgHV1-69-04 germline; and
- c) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs:131, 132, and 133, such as the CRD1, CDR2, and CDR3 sequences of SEQ ID NOs: 2, 3 and 4 (169), respectively, optionally where the VH region is derived from an IgHV1-18-1 germline.

In one embodiment, the antibody comprises a VH region selected from the preceding embodiments (a) or (b) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:13, XAS (wherein X is A or V), and SEQ ID No:155, respectively, such as a CDR1 sequence selected from SEQ ID Nos: 13 or 20, a CDR2 which is AAS or VAS, and a

CDR3 sequence selected from SEQ ID NOs:14 and 21 (**050, 084**); respectively, optionally where the VL region is derived from an IgKV1-12-01 germline.

In one embodiment, the antibody comprises a VH region which is the preceding embodiment (c) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:6, DXS (wherein X=A or T), and SEQ ID NO:156 (**169**), respectively, optionally wherein the VL region is derived from IgKV3-11-01.

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:2, 3 and 4, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:6, DAS, and SEQ ID NO:7, respectively (**169**).

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:9, 10 and 11, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:13, AAS, and SEQ ID NO:14, respectively (**050**).

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:16, 17 and 18, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:20, VAS, and SEQ ID NO:21, respectively (**084**).

In separate embodiments, the antibody comprises:

- a) a VH region comprising the sequence of SEQ ID NO:1 and, preferably, a VL region comprising the sequence of SEQ ID NO:5 (**169**);
- b) a VH region comprising the sequence of SEQ ID NO:8 and, preferably, a VL region comprising the sequence of SEQ ID NO:12 (**050**);
- c) a VH region comprising the sequence of SEQ ID NO:15 and, preferably, a VL region comprising the sequence of SEQ ID NO:19 (**084**);
- d) a VH region comprising the sequence of SEQ ID NO:77 and, preferably, a VL region comprising the sequence of SEQ ID NO:78 (**049**);
- e) a VH region comprising the sequence of SEQ ID NO:79 and, preferably, a VL region comprising the sequence of SEQ ID NO:80 (**051**);
- f) a VH region comprising the sequence of SEQ ID NO:81 and, preferably, a VL region comprising the sequence of SEQ ID NO:82 (**055**);
- g) a VH region comprising the sequence of SEQ ID NO:83 and, preferably, a VL region comprising the sequence of SEQ ID NO:84 (**123**);

- h) a VH region comprising the sequence of SEQ ID NO:85 and, preferably, a VL region comprising the sequence of SEQ ID NO:86 (161);
- i) a VH region comprising the sequence of SEQ ID NO:87 and, preferably, a VL region comprising the sequence of SEQ ID NO:88 (124); and/or
- j) a variant of any of said antibodies, wherein said variant preferably has at most 1, 2 or 3 amino-acid modifications, more preferably amino-acid substitutions, such as conservative amino acid substitutions and substitutions where the new amino acid is one at the same position in an aligned sequence in Figures 1 or 2, particularly at positions indicated by "X" in the corresponding consensus sequence.

Antibodies of cross-block group 2

In one aspect of the antibody of the invention, the antibody binds the same epitope on HER2 as one or more of the novel human antibodies of cross-block group 2 described herein.

In one embodiment, the antibody cross-blocks the binding to soluble HER2 of pertuzumab, when determined as described in Example 14.

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:22 and a VL region comprising the sequence of SEQ ID NO:26 (025).

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:32 (091).

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:35 and a VL region comprising the sequence of SEQ ID NO:39 (129).

In one embodiment, the antibody binds to the same epitope as a reference antibody comprising VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:89 and a VL region comprising the sequence of SEQ ID NO:90 (001);
- b) a VH region comprising the sequence of SEQ ID NO:91 and a VL region comprising the sequence of SEQ ID NO:92 (143);
- c) a VH region comprising the sequence of SEQ ID NO:93 and a VL region comprising the sequence of SEQ ID NO:94 (019);
- d) a VH region comprising the sequence of SEQ ID NO:95 and a VL region comprising the sequence of SEQ ID NO:96 (021);

- e) a VH region comprising the sequence of SEQ ID NO:97 and a VL region comprising the sequence of SEQ ID NO:98 (027);
- f) a VH region comprising the sequence of SEQ ID NO:99 and a VL region comprising the sequence of SEQ ID NO:100 (032)
- g) a VH region comprising the sequence of SEQ ID NO:101 and a VL region comprising the sequence of SEQ ID NO:102 (035);
- h) a VH region comprising the sequence of SEQ ID NO:103 and a VL region comprising the sequence of SEQ ID NO:104 (036);
- i) a VH region comprising the sequence of SEQ ID NO:105 and a VL region comprising the sequence of SEQ ID NO:106 (054); and
- j) a VH region comprising the sequence of SEQ ID NO:107 and a VL region comprising the sequence of SEQ ID NO:108 (094).

In another additional or alternative aspect of the antibody of the invention, the antibody binds to HER2 and comprises a VH CDR3, VH region and/or VL region sequence similar or identical to a sequence of the novel antibodies described herein.

In one embodiment, the antibody comprises a VH CDR3 region having a sequence selected from the group consisting of

SEQ ID NO:136, such as the sequence of SEQ ID NO:25 (025), optionally wherein the VH region is derived from the IgHV4-34-1 germline sequence;

SEQ ID NO:139, such as the sequence of SEQ ID NO:31 (091), optionally wherein the VH region is derived from the IgHV4-34-01 germline sequence; and

SEQ ID NO:142, such as the sequence of SEQ ID NO:38 (129), optionally wherein the VH region is derived from the IgHV3-30-01 germline sequence.

In one embodiment, the antibody comprises a VH CDR3 region of one of antibodies 001, 143, 019, 021, 027, 032, 035, 036, 054 or 094 as shown in Figure 1, optionally wherein the VH region is derived from an IgHV4-34-1 germline.

In one embodiment, the antibody comprises a VH region selected from the group consisting of

- a) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:134, 135 and 136, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID NOS: 23, 24 and 25 (025); optionally where the VH region is derived from an IgHV4-34-1 germline;

- b) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:137, 138 and 139, such the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:30, 163, and 31, respectively (**091**), optionally where the VH region is derived from an IgHV4-34-01 germline; and
- c) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs:140, 141 and 142, such as the CRD1, CDR2, and CDR3 sequences of SEQ ID NOs: 36, 37 and 38 (**129**), respectively, optionally where the VH region is derived from an IgHV3-30-01 germline.

In one embodiment, the antibody comprises a VH region selected from the preceding embodiment (a) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:157, AAS, and SEQ ID No:164, respectively, such as the CDR1, CDR2, and CDR3 sequences of SEQ ID Nos:27, AAS, and SEQ ID NO:28 (**025**); respectively, optionally where the VL region is derived from an IgKV1D-16-01 germline.

In one embodiment, the antibody comprises a VH region selected from the preceding embodiment (b) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:33, AX<sub>1</sub>X<sub>2</sub> (wherein X<sub>1</sub> is A or T, preferably A; and X<sub>2</sub> is S or F, preferably S), and SEQ ID No:158, respectively, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID Nos:33, AAS, and SEQ ID NO:34 (**091**); respectively, optionally where the VL region is derived from an IgKV1D-16-01 germline.

In one embodiment, the antibody comprises a VH region which is the preceding embodiment (c) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:40, DAS and SEQ ID NO:41 (**129**), respectively, optionally wherein the VL region is derived from IgKV3-11-01.

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:23, 24 and 25, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:27, AAS, and SEQ ID NO:28, respectively (**025**).

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:30, 163 and 31, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:33, AAS, and SEQ ID NO:34, respectively (**091**).

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:36, 37 and 38, respectively; and a VL region

comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:40, DAS, and SEQ ID NO:41, respectively (129).

In separate embodiments, the antibody comprises:

- a) a VH region comprising the sequence of SEQ ID NO:22 and, preferably, a VL region comprising the sequence of SEQ ID NO:26 (025);
- b) a VH region comprising the sequence of SEQ ID NO:29 and, preferably, a VL region comprising the sequence of SEQ ID NO:32 (091);
- c) a VH region comprising the sequence of SEQ ID NO:35 and, preferably, a VL region comprising the sequence of SEQ ID NO:39 (129);
- d) a VH region comprising the sequence of SEQ ID NO:89 and, preferably, a VL region comprising the sequence of SEQ ID NO:90 (001);
- e) a VH region comprising the sequence of SEQ ID NO:91 and, preferably, a VL region comprising the sequence of SEQ ID NO:92 (143);
- f) a VH region comprising the sequence of SEQ ID NO:93 and, preferably, a VL region comprising the sequence of SEQ ID NO:94 (019);
- g) a VH region comprising the sequence of SEQ ID NO:95 and, preferably, a VL region comprising the sequence of SEQ ID NO:96 (021);
- h) a VH region comprising the sequence of SEQ ID NO:97 and, preferably, a VL region comprising the sequence of SEQ ID NO:98 (027);
- i) a VH region comprising the sequence of SEQ ID NO:99 and, preferably, a VL region comprising the sequence of SEQ ID NO:100 (032);
- j) a VH region comprising the sequence of SEQ ID NO:101 and, preferably, a VL region comprising the sequence of SEQ ID NO:102 (035);
- k) a VH region comprising the sequence of SEQ ID NO:103 and, preferably, a VL region comprising the sequence of SEQ ID NO:104 (036);
- l) a VH region comprising the sequence of SEQ ID NO:105 and, preferably, a VL region comprising the sequence of SEQ ID NO:106 (054);
- m) a VH region comprising the sequence of SEQ ID NO:106 and, preferably, a VL region comprising the sequence of SEQ ID NO:108 (094); and/or
- n) a variant of any of said antibodies, wherein said variant preferably has at most 1, 2 or 3 amino-acid modifications, more preferably amino-acid substitutions, such as conservative amino acid substitutions and substitutions where the new amino acid is one at the same position in an aligned sequence in Figures 1 or 2, particularly at positions indicated by "X" in the corresponding consensus sequence.

Antibodies of cross-block group 3

In one aspect of the antibody of the invention, the antibody binds the same epitope on HER2 as one or more of the novel human antibodies of cross-block group 3 described herein.

In one embodiment, the antibody cross-blocks the binding to soluble HER2 of F5 and/or C5, when determined as described in Example 14.

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:46 and a VL region comprising the sequence of SEQ ID NO:49 (**127**).

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:49 and a VL region comprising the sequence of SEQ ID NO:53 (**159**).

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:56 and a VL region comprising the sequence of SEQ ID NO:60 (**098**).

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:63 and a VL region comprising the sequence of SEQ ID NO:67 (**153**).

In one embodiment, the antibody binds the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:70 and a VL region comprising the sequence of SEQ ID NO:74 (**132**).

In one embodiment, the antibody binds to the same epitope as a reference antibody comprising VH and VL regions selected from the group consisting of:

- k) a VH region comprising the sequence of SEQ ID NO:109 and a VL region comprising the sequence of SEQ ID NO:110 (**105**);
- l) a VH region comprising the sequence of SEQ ID NO:111 and a VL region comprising the sequence of SEQ ID NO:112 (**100**);
- m) a VH region comprising the sequence of SEQ ID NO:113 and a VL region comprising the sequence of SEQ ID NO:114 (**125**);
- n) a VH region comprising the sequence of SEQ ID NO:115 and a VL region comprising the sequence of SEQ ID NO:116 (**162**);
- o) a VH region comprising the sequence of SEQ ID NO:117 and a VL region comprising the sequence of SEQ ID NO:118 (**033**);
- p) a VH region comprising the sequence of SEQ ID NO:119 and a VL region comprising the sequence of SEQ ID NO:120 (**160**)

- q) a VH region comprising the sequence of SEQ ID NO:121 and a VL region comprising the sequence of SEQ ID NO:122 (166);
- r) a VH region comprising the sequence of SEQ ID NO:123 and a VL region comprising the sequence of SEQ ID NO:124 (152); and
- s) a VH region comprising the sequence of SEQ ID NO:125 and a VL region comprising the sequence of SEQ ID NO:126 (167).

In another additional or alternative aspect of the antibody of the invention, the antibody binds to HER2 and comprises a VH CDR3, VH region and/or VL region sequence similar or identical to a sequence of the novel antibodies described herein.

In one embodiment, the antibody comprises a VH CDR3 region having a sequence selected from the group consisting of

SEQ ID NO:148, such as the sequence of SEQ ID NO:48 (127), optionally wherein the VH region is derived from the IgHV5-51-01 germline sequence;

SEQ ID NO:52 (159), optionally wherein the VH region is derived from the IgHV5-51-01 germline sequence;

SEQ ID NO:145, such as the sequence of SEQ ID NO:59 (098), optionally wherein the VH region is derived from the IgHV3-23-01 germline sequence;

SEQ ID NO:154, such as the sequence of SEQ ID NO:66 (153), optionally wherein the VH region is derived from the IgHV3-30-03-01 germline sequence; and

SEQ ID NO:151, such as the sequence of SEQ ID NO:73 (132), optionally wherein the VH region is derived from the IgHV1-18-01 germline sequence.

In one embodiment, the antibody comprises a VH CDR3 region of one of antibodies 105, 100, 125 or 162 as shown in Figure 1, optionally wherein the VH region is derived from an IgHV3-23-1 germline.

In one embodiment, the antibody comprises a VH CDR3 region of one of antibodies 033, 160, 166, 152 or 167 as shown in Figure 1, optionally wherein the VH region is derived from an IgHV3-30-3-01 germline.

In one embodiment, the antibody comprises a VH region selected from the group consisting of

- a) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:146, 147 and 148, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID NOS: 43, 44 and 45 (127); optionally where the VH region is derived from an IgHV5-51-01 germline;

- b) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:149, 51 and 52, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:50, 51 and 52, respectively (**159**), optionally where the VH region is derived from an IgHV5-51-01 germline;
- c) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs:143, 144 and 145, such as the CRD1, CDR2, and CDR3 sequences of SEQ ID NOs: 57, 58 and 59 (**098**), respectively, optionally where the VH region is derived from an IgHV3-23-01 germline;
- d) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:152, 153 and 154, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:64, 65 and 66, respectively (**153**), optionally where the VH region is derived from an IgHV3-30-03-01 germline; and
- e) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs:71, 150 and 151, such as the CRD1, CDR2, and CDR3 sequences of SEQ ID NOs: 71, 72 and 73 (**132**), respectively, optionally where the VH region is derived from an IgHV1-18-01 germline.

In one embodiment, the antibody comprises a VH region selected from the preceding embodiment (a) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:47, AAS and SEQ ID NO:48, respectively (**127**); respectively, optionally where the VL region is derived from an IgKV1D-8-01 germline.

In one embodiment, the antibody comprises a VH region selected from the preceding embodiment (b) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:54, AAS, and SEQ ID No:55 (**159**); respectively, optionally where the VL region is derived from an IgKV1D-16-01 germline.

In one embodiment, the antibody comprises a VH region which is the preceding embodiment (c) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:159, AAS and SEQ ID NO:160, respectively, such as the VL CDR1, CDR2 and CDR3 sequences of SEQ ID NOS: 61, AAS and SEQ ID NO:62 (**098**), optionally wherein the VL region is derived from IgKV1D-16-01.

In one embodiment, the antibody comprises a VH region which is the preceding embodiment (d) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:161, XAS (wherein X=D or A, preferably D), and SEQ ID NO:162 (**153**), respectively, such as the VL CDR sequences of SEQ ID NO:68, DAS, and 69, optionally wherein the VL region is derived from IgKV1D-16-01.

In one embodiment, the antibody comprises a VH region which is the preceding embodiment (e) and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:75, DAS and SEQ ID NO:76 (132), respectively, optionally wherein the VL region is derived from IgKV3-11-01.

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:43, 44 and 45, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:47, AAS, and SEQ ID NO:48, respectively (127).

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:50, 51 and 52, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:54, AAS, and SEQ ID NO:55, respectively (159).

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:57, 58 and 59, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:60, AAS, and SEQ ID NO:61, respectively (098).

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:64, 65 and 66, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:68, DAS, and SEQ ID NO:69, respectively (153).

In one embodiment, the antibody comprises a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:71, 72 and 73, respectively; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:75, DAS, and SEQ ID NO:76, respectively (132).

In separate embodiments, the antibody comprises:

- a) a VH region comprising the sequence of SEQ ID NO:46 and, preferably, a VL region comprising the sequence of SEQ ID NO:49 (127);
- b) a VH region comprising the sequence of SEQ ID NO:49 and, preferably, a VL region comprising the sequence of SEQ ID NO:53 (159);
- c) a VH region comprising the sequence of SEQ ID NO:56 and, preferably, a VL region comprising the sequence of SEQ ID NO:60 (098);
- d) a VH region comprising the sequence of SEQ ID NO:63 and, preferably, a VL region comprising the sequence of SEQ ID NO:67 (153);

- e) a VH region comprising the sequence of SEQ ID NO:70 and, preferably, a VL region comprising the sequence of SEQ ID NO:74 (**132**);
- f) a VH region comprising the sequence of SEQ ID NO:109 and, preferably, a VL region comprising the sequence of SEQ ID NO:110 (**105**);
- g) a VH region comprising the sequence of SEQ ID NO:111 and, preferably, a VL region comprising the sequence of SEQ ID NO:112 (**100**);
- h) a VH region comprising the sequence of SEQ ID NO:113 and, preferably, a VL region comprising the sequence of SEQ ID NO:114 (**125**);
- i) a VH region comprising the sequence of SEQ ID NO:115 and, preferably, a VL region comprising the sequence of SEQ ID NO:116 (**162**);
- j) a VH region comprising the sequence of SEQ ID NO:117 and, preferably, a VL region comprising the sequence of SEQ ID NO:118 (**033**);
- k) a VH region comprising the sequence of SEQ ID NO:119 and, preferably, a VL region comprising the sequence of SEQ ID NO:120 (**160**);
- l) a VH region comprising the sequence of SEQ ID NO:121 and, preferably, a VL region comprising the sequence of SEQ ID NO:122 (**166**);
- m) a VH region comprising the sequence of SEQ ID NO:123 and, preferably, a VL region comprising the sequence of SEQ ID NO:124 (**152**);
- o) a VH region comprising the sequence of SEQ ID NO:125 and, preferably, a VL region comprising the sequence of SEQ ID NO:126 (**167**); and/or
- p) a variant of any of said antibodies, wherein said variant preferably has at most 1, 2 or 3 amino-acid modifications, more preferably amino-acid substitutions, such as conservative amino acid substitutions and substitutions where the new amino acid is one at the same position in an aligned sequence in Figures 1 or 2, particularly at positions indicated by "X" in the corresponding consensus sequence.

#### Bispecific antibodies

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody comprising antigen-binding region of an antibody as defined herein, e.g. an antibody of cross-block 1, 2 or 3, or the VH and VL region comprising the sequences of (**005**), and (ii) a second antibody comprising an antigen-binding region of an antibody which binds to CD3.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody comprising an antigen-binding region of an antibody as defined herein or the VH and VL region comprising the sequences of (**005**), and (ii) a second antibody comprising antigen-binding region of an antibody as defined herein or the VH and VL region comprising

sequences of (005), wherein the first antigen-binding region binds to a different epitope than the second antigen-binding region.

In one embodiment the first antibody comprises a VH region comprising a CDR3 sequence of an antibody of cross-block 1, 2 or 3 as defined herein, such as SEQ ID NO: 4, 25 or 66 (169, 025 or 153), or a CDR3 sequence of SEQ ID NO:168 (005).

In one embodiment the first antibody comprises a VH region comprising CDR1, CDR2 and CDR3 sequences of an antibody of cross-block 1, 2 or 3 as defined herein, such as CDR1, CDR2, and CDR3 sequences SEQ ID NOs: 2, 3 and 4 (169), or CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:23, 24 and 25 (025), or CDR1, CDR2 and CDR3 sequences of SEQ ID NOs: 64, 65 and 66 (153), or CDR1, CDR2 CDR3 sequence of SEQ ID NOs: 166, 167 and 168 (005).

In a further or alternative embodiment the first antibody comprises a VH region comprising a CDR3 sequence of an antibody of cross-block 1, 2 or 3 as defined herein, such as CDR3 sequence an antibody of cross-block 1 of SEQ ID NO: 11 (050), or SEQ ID NO: 18 (084); or a CDR3 sequence of an antibody of cross-block 2 of SEQ ID NO: 31 (091), or SEQ ID NO: 38 (129), or a CDR3 sequence of an antibody of cross-block 3 of SEQ ID NO: 45 (127), or SEQ ID NO:52 (159), or SEQ ID NO:59 (098), or SEQ ID NO:73 (132).

In one embodiment the first antibody comprises a VH region comprising CDR1, CDR2 and CDR3 sequences of an antibody of cross-block 1, 2 or 3 as defined herein, such as CDR1, CDR2, and CDR3 sequences SEQ ID NOs: 2, 3 and 4 (169), or CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:23, 24 and 25 (025), or CDR1, CDR2 and CDR3 sequences of SEQ ID NOs: 64, 65 and 66 (153), or CDR1, CDR2 CDR3 sequence of SEQ ID NOs: 166, 167 and 168 (005).

In one embodiment the first antibody comprises a VH region comprising CDR1, CDR2 and CDR3 sequences of an antibody of cross-block 1, 2 or 3 as defined herein a VL region comprising CDR1, CDR2 and CDR3 sequences of an antibody of cross-block 1, 2 or 3 as defined herein.

In a further or alternative embodiment the first antibody comprises a VH region comprising CDR1, CDR2 and CDR3 sequences of an antibody of cross-block 1, 2 or 3 as defined herein, such as CDR1, CDR2, and CDR3 sequences of an antibody of cross-block 1 of SEQ ID NOs: 9, 10 and 11 (050), or SEQ ID NOs: 16, 17 and 18 (084); or CDR1, CDR2, and CDR3 sequences of an antibody of cross-block 2 of SEQ ID NOs: 30, 163 and 31 (091), or SEQ ID NOs: 36, 37 and 38 (129), or CDR1, CDR2, and CDR3 sequences of an antibody of cross-block 3 SEQ ID NOs: 43, 44 and 45 (127), or SEQ ID NOs:50, 51 and 52 (159), or SEQ ID NOs:57, 58 and 59 (098), or SEQ ID NOs:71, 72 and 73 (132).

In one embodiment the first antibody comprises a VH region and a VL region selected from the group consisting of:

- a) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 2, 3 and 4; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID: 6, GAS and SEQ ID NO:7, respectively (**169**);
- b) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs: 23, 24 and 25; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NO: 27, AAS and SEQ ID NO:28, respectively (**025**);
- c) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:64, 65 and 66; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NO: 68, DAS and SEQ ID NO:69 (**153**); and
- d) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:166, 167 and 168; and a VL region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NO: 169, GAS and SEQ ID NO:170 (**005**).

In a further or alternative embodiment the first antibody comprises a VH region and a VL region selected from the group consisting of:

- a) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:9, 127 and 11, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID NOS: 9, 10 and 11 (**050**); optionally where the VH region is derived from an IgHV3-23-1 germline;
- b) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:128, 129 and 130, such the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:16, 17 and 18, respectively (**084**), optionally where the VH region is derived from an IgHV1-69-04 germline; and
- c) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:137, 138 and 139, such the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:30, 163, and 31, respectively (**091**), optionally where the VH region is derived from an IgHV4-34-01 germline; and
- d) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs:140, 141 and 142, such as the CRD1, CDR2, and CDR3 sequences of SEQ ID NOs: 36, 37 and 38 (**129**), respectively, optionally where the VH region is derived from an IgHV3-30-01 germline.
- e) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:146, 147 and 148, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID NOS: 43, 44 and 45 (**127**); optionally where the VH region is derived from an IgHV5-51-01 germline;

- f) a VH region comprising the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:149, 51 and 52, such as the CDR1, CDR2 and CDR3 sequences of SEQ ID NOs:50, 51 and 52, respectively (**159**), optionally where the VH region is derived from an IgHV5-51-01 germline;
- g) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs:143, 144 and 145, such as the CRD1, CDR2, and CDR3 sequences of SEQ ID NOs: 57, 58 and 59 (**098**), respectively, optionally where the VH region is derived from an IgHV3-23-01 germline;
- h) a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOs:71, 150 and 151, such as the CRD1, CDR2, and CDR3 sequences of SEQ ID NOs: 71, 72 and 73 (**132**), respectively, optionally where the VH region is derived from an IgHV1-18-01 germline.

In one embodiment the second antibody is one of the previous embodiment described for the first antibody, but wherein the second antibody binds to a different epitope than the first antibody.

In one embodiment the second antibody is a CD3 antibody. In one embodiment a CD3 antibody may be an antibody comprising a VH region comprising the sequence of SEQ ID NO: 171 (**YTH12.5**) and VL region comprising the sequence of SEQ ID NO:172 (**YTH12.5**). Another example of a CD3 antibody is an antibody comprising a VH region comprising the sequence of SEQ ID NO: 173 (**huCLB-T3/ 4**) and VL region comprising the sequence of SEQ ID NO:174 (**huCLB-T3/ 4**).

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, which antibody comprises the VH and VL region sequences of (**005**), (**025**), (**153**) or (**169**), and which antibody comprises an IgG1 wildtype Fc region, wherein the CH3 region contains Ile at position 350, a Thr at position 370, and a Leu at position 405 and (ii) a second antibody having an Fc region and VH and VL sequences, which antibody comprising the VH and VL region sequences of (**005**), (**025**), (**153**) or (**169**), and which antibody comprises a IgG1 wildtype Fc region, wherein the CH3 region contains an Arg at position 409. Specific embodiments are disclosed in examples.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO: 164, and the VL region comprises the amino acid sequence of SEQ ID NO: 165 (**005**), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region, wherein the CH3 region contains Ile at position 350, a Thr at position 370,

and a Leu at position 405; and (ii) a second antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:1 and the VL region comprises the amino acid sequence of SEQ ID NO:5 (169), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Arg at position 409.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:22, and the VL region comprises the amino acid sequence of SEQ ID NO:26 (025), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region, wherein the CH3 region contains Ile at position 350, a Thr at position 370, and a Leu at position 405; and (ii) a second antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:164 and the VL region comprises the amino acid sequence of SEQ ID NO:165 (005), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Arg at position 409.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:22, and the VL region comprises the amino acid sequence of SEQ ID NO:26 (025), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region, wherein the CH3 region contains Ile at position 350, a Thr at position 370, and a Leu at position 405; and (ii) a second antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:63 and the VL region comprises the amino acid sequence of SEQ ID NO:37 (153), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Arg at position 409.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:22, and the VL region comprises the amino acid sequence of SEQ ID NO:26 (025), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region, wherein the CH3 region contains Ile at position 350, a Thr at position 370, and a Leu at position 405; and (ii) a second antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:1 and the VL region comprises the amino acid sequence of SEQ ID NO:5 (169), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Arg at position 409.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:63, and the VL region comprises the amino acid sequence of SEQ ID NO:67 (153), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region, wherein the CH3 region contains Ile at position 350, a Thr at position 370, and a

Leu at position 405; and (ii) a second antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:164 and the VL region comprises the amino acid sequence of SEQ ID NO:165 (005), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Arg at position 409.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:63, and the VL region comprises the amino acid sequence of SEQ ID NO:67 (153), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region, wherein the CH3 region contains Ile at position 350, a Thr at position 370, and a Leu at position 405; and (ii) a second antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:1 and the VL region comprises the amino acid sequence of SEQ ID NO:5 (169), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Arg at position 409.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:63, and the VL region comprises the amino acid sequence of SEQ ID NO:67 (153), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region having Arg at position 409, or Gln at position 297, or Arg at position 409 and Gln at position 297; and (ii) a second antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:171 and the VL region comprises the amino acid sequence of SEQ ID NO:172 (YTH12.5), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Gln at position 297, or Leu at position 405, or Gln at position 297 and Leu at position 405.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:1, and the VL region comprises the amino acid sequence of SEQ ID NO:5 (169), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region having Arg at position 409; and (ii) a second antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:171 and the VL region comprises the amino acid sequence of SEQ ID NO:172 (YTH12.5), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Gln at position 297, or Leu at position 405, or Gln at position 297 and Leu at position 405.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:63, and the VL region comprises the amino acid

sequence of SEQ ID NO:67 (**153**), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region having Arg at position 409, or Gln at position 297, or Arg at position 409 and Gln at position 297; and (ii) a second antibody having an Fc region and VH and VL sequences ,wherein the VH region comprises the amino acid sequence of SEQ ID NO:173 and the VL region comprises the amino acid sequence of SEQ ID NO:174 (huCLB-T3/4), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Gln at position 297, or Leu at position 405, or Gln at position 297 and Leu at position 405.

In one embodiment, the antibody is a bispecific antibody, comprising (i) a first antibody having an Fc region and VH and VL sequences, wherein the VH region comprises the amino acid sequence of SEQ ID NO:1, and the VL region comprises the amino acid sequence of SEQ ID NO:5 (**169**), optionally wherein the first antibody comprises an IgG1, $\kappa$  Fc region having Arg at position 409; and (ii) a second antibody having an Fc region and VH and VL sequences ,wherein the VH region comprises the amino acid sequence of SEQ ID NO:173 and the VL region comprises the amino acid sequence of SEQ ID NO:174 (huCLB-T3/4), optionally wherein the second antibody comprises an IgG1, $\kappa$  Fc region having an Gln at position 297, or Leu at position 405, or Gln at position 297 and Leu at position 405.

A CD3 antibody is an antibody with a VH region comprising the sequence of SEQ ID NO: 171 (VH YTH12.5) and VL region comprising the sequence of SEQ ID NO:172 (VL YTH12.5). Another example is a CD3 antibody with a VH region comprising the sequence of SEQ ID NO: 173 (VH huCLB-T3/4) and VL region comprising the sequence of SEQ ID NO:174 (VL huCLB-T3/4).

In one embodiment a bispecific antibody of the present invention may be selected from the group consisting of: IgG1-005-ITL x IgG1-169-K409R, IgG1-025-ITL x IgG1-005-K409R, IgG1-025-ITL x IgG1-153-K409R, IgG1-025-ITL x IgG1-169-K409R, IgG1-153-ITL x IgG1-005-K409R; and IgG1-153-ITL x IgG1-169-K409R, wherein IgG1-005-ITL means **005** IgG1, $\kappa$  having Ile at position 350, Thr at position 370, and Leu at position 405, IgG1-005-K409R means **005** IgG1, $\kappa$  having an Arg at position 409, IgG1-025-ITL means **025** IgG1, $\kappa$  having Ile at position 350, Thr at position 370, and Leu at position 405, IgG1-153-ITL means **153** IgG1, $\kappa$  having contains Ile at position 350, Thr at position 370, and Leu at position 405, IgG1-153-K409R means **153** IgG1, $\kappa$  having an Arg at position 409, IgG1-169-K409R means **169** IgG1, $\kappa$  having an Arg at position 409, and wherein the bold numbers refer to antibodies described herein with the VH and VL regions comprising the sequences described in table 1 and for **005** in example 21; i.e. SEQ ID NOs:164 and 165.

In one embodiment the bispecific antibody may be selected from the group consisting of: IgG1-HER2-153-K409R x IgG1-YTH12.5-F405L, IgG1-HER2-153-K409R x IgG1-YTH12.5-N297Q-F405L, IgG1-HER2-153-K409R x IgG1-hu-CLB-T3/4-F405L, IgG1-

HER2-153-K409R x IgG1-hu-CLB-T3/4-N297Q-F405L, IgG1-HER2-153-N297Q-K409R x IgG1-YTH12.5-F405L, IgG1-HER2-153-N297Q-K409R x IgG1-YTH12.5-N297Q-F405L, IgG1-HER2-153-N297Q-K409R x IgG1-hu-CLB-T3/4-F405L, IgG1-HER2-153-N297Q-K409R x IgG1-hu-CLB-T3/4-N297Q-F405L, IgG1-HER2-169-K409R x IgG1-hu-CLB-T3/4-F405L, IgG1-HER2-169-K409R x IgG1-hu-CLB-T3/4-N297Q-F405L, IgG1-HER2-169-K409R x IgG1-YTH12.5-F405L and IgG1-HER2-169-K409R x IgG1-YTH12.5-N297Q-F405L.

Functional properties of Group 1, 2 and 3 antibodies and bispecific antibodies

In another aspect of the antibody of the invention, the antibody binds to the same HER2 epitope as one or more of the novel Group 1, 2 or 3 antibodies described herein, preferably when determined as described in Example 14; and is further characterized by one or more properties determined as described in Examples 12, 13, 15, 16, 17, 18 and 19.

In one embodiment, the HER2 antibody has a lower EC<sub>50</sub> value (half maximal effective concentration) than trastuzumab in binding to A431 cells, preferably an EC<sub>50</sub> value lower than 0.80 µg/ml, 0.50 µg/ml, or 0.30 µg/ml, when determined as described in Example 12, and preferably binds the same epitope as at least one reference antibody comprising the VH and VL regions selected from the group consisting of

- a) a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5 (**169**);
- b) a VH region comprising the sequence of SEQ ID NO:15 and a VL region comprising the sequence of SEQ ID NO:19 (**084**);
- c) a VH region comprising the sequence of SEQ ID NO:22 and a VL region comprising the sequence of SEQ ID NO:26 (**025**);
- d) a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:32 (**091**);
- e) a VH region comprising the sequence of SEQ ID NO:46 and a VL region comprising the sequence of SEQ ID NO:49 (**127**);
- f) a VH region comprising the sequence of SEQ ID NO:49 and a VL region comprising the sequence of SEQ ID NO:53 (**159**);
- g) a VH region comprising the sequence of SEQ ID NO:56 and a VL region comprising the sequence of SEQ ID NO:60 (**098**);
- h) a VH region comprising the sequence of SEQ ID NO:63 and a VL region comprising the sequence of SEQ ID NO:67 (**153**); and
- i) a VH region comprising the sequence of SEQ ID NO:70 and a VL region comprising the sequence of SEQ ID NO:74 (**132**).

In an additional or alternative embodiment, the anti-HER2 antibody specifically binds HER2-positive Rhesus epithelial cells, when determined as described in Example 13, and preferably binds the same epitope as at least one reference antibody comprising the VH and VL regions selected from the group consisting of the VH and VL regions of any of antibodies 169, 050, 084, 025, 091, 129, 127, 159, 098, 153 and 132.

In an additional or alternative embodiment, the anti-HER2 antibody efficiently induces ADCC (antibody-dependent cell-mediated cytotoxicity), preferably achieving a specific <sup>51</sup>Cr-release of at least 30%, more preferably at least 40%, when determined as described in Example 15, and preferably binds the same epitope as at least one reference antibody comprising the VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5 (**169**);
- b) a VH region comprising the sequence of SEQ ID NO:8 and a VL region comprising the sequence of SEQ ID NO:12 (**050**);
- c) a VH region comprising the sequence of SEQ ID NO:15 and a VL region comprising the sequence of SEQ ID NO:19 (**084**);
- d) a VH region comprising the sequence of SEQ ID NO:22 and a VL region comprising the sequence of SEQ ID NO:26 (**025**);
- e) a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:32 (**091**);
- f) a VH region comprising the sequence of SEQ ID NO:35 and a VL region comprising the sequence of SEQ ID NO:39 (**129**); and
- g) a VH region comprising the sequence of SEQ ID NO:63 and, preferably, a VL region comprising the sequence of SEQ ID NO:67 (**153**).

In an additional or alternative embodiment, the HER2 antibody specifically binds HER2-expressing AU565 cells but promotes ligand-independent proliferation of the cells less than any of F5 and C1 when determined as described in Example 16, and preferably binds the same epitope as at least one reference antibody comprising the VH and VL regions selected from the group consisting of

- a) a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5 (**169**);
- b) a VH region comprising the sequence of SEQ ID NO:8 and a VL region comprising the sequence of SEQ ID NO:12 (**050**);

- c) a VH region comprising the sequence of SEQ ID NO:15 and a VL region comprising the sequence of SEQ ID NO:19 (**084**);
- d) a VH region comprising the sequence of SEQ ID NO:22 and a VL region comprising the sequence of SEQ ID NO:26 (**025**);
- e) a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:32 (**091**);
- f) a VH region comprising the sequence of SEQ ID NO:35 and a VL region comprising the sequence of SEQ ID NO:39 (**129**);
- g) a VH region comprising the sequence of SEQ ID NO:46 and a VL region comprising the sequence of SEQ ID NO:49 (**127**);
- h) a VH region comprising the sequence of SEQ ID NO:49 and a VL region comprising the sequence of SEQ ID NO:53 (**159**);
- i) a VH region comprising the sequence of SEQ ID NO:56 and a VL region comprising the sequence of SEQ ID NO:60 (**098**);
- j) a VH region comprising the sequence of SEQ ID NO:63 and a VL region comprising the sequence of SEQ ID NO:67 (**153**); and
- k) a VH region comprising the sequence of SEQ ID NO:70 and a VL region comprising the sequence of SEQ ID NO:74 (**132**).

In an additional or alternative embodiment, the HER2 antibody specifically binds HER2-expressing AU565 cells and inhibits ligand-independent proliferation of the cells, preferably inhibiting proliferation by at least 20% more preferably at least 25% when determined as described in Example 16, and preferably binds the same epitope as at least one reference antibody comprising the VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5 (**169**); and
- b) a VH region comprising the sequence of SEQ ID NO:8 and a VL region comprising the sequence of SEQ ID NO:12 (**050**).

In an additional or alternative embodiment, the HER2 antibody specifically binds HER2-expressing AU565 cells but has no significant effect on, or does not promote, ligand-induced proliferation of the cells, preferably inhibiting proliferation by no more than 25%, more preferably by no more than 15%, when determined as described in Example 17, and binds the same epitope as at least one reference antibody comprising the VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5 (**169**);
- b) a VH region comprising the sequence of SEQ ID NO:8 and a VL region comprising the sequence of SEQ ID NO:12 (**050**);
- c) a VH region comprising the sequence of SEQ ID NO:15 and a VL region comprising the sequence of SEQ ID NO:19 (**084**); and
- d) a VH region comprising the sequence of SEQ ID NO:56 and a VL region comprising the sequence of SEQ ID NO:60 (**098**).

In an additional or alternative embodiment, the HER2 antibody specifically binds HER2-expressing MCF-7 cells and inhibits ligand-induced proliferation, e.g. it may completely inhibit the ligand-induced effect or inhibit the total proliferation by 50%, e.g. 60% or 70% or 80%, of the cells when determined as described in Example 17, and binds the same epitope as at least one reference antibody comprising the VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:22 and a VL region comprising the sequence of SEQ ID NO:26 (**025**);
- b) a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:32 (**091**);
- c) a VH region comprising the sequence of SEQ ID NO:35 and a VL region comprising the sequence of SEQ ID NO:39 (**129**); and
- d) a VH region comprising the sequence of SEQ ID NO:63 and, preferably, a VL region comprising the sequence of SEQ ID NO:67 (**153**).

In an additional or alternative embodiment, the antibody, when conjugated directly or indirectly to a therapeutic moiety such as a truncated form of the pseudomonas-exotoxin A, is more effective than trastuzumab in killing AU565 cells, A431 cells, or both AU565 and A431 cells, when determined as described in Example 18.

In one embodiment, the conjugated antibody has an EC<sub>50</sub> value of less than 70 ng/ml, less than 50 ng/ml, or less than 30 ng/ml in killing AU565 cells and/or A431 cells, when determined as described in Example 18, and binds the same epitope as at least one reference antibody comprising the VH and VL regions of an antibody selected from the group consisting of 169, 091, 050, 084, 098, 05, 153, 129, 132, 127 and 159; preferably selected from antibodies 153, 129, 098, 091 and 025.

In one embodiment, the conjugated antibody has or results in a higher percentage of killed AU565 cells than trastuzumab and pertuzumab when determined as described in

Example 18, preferably killing at least 49%, more preferably at least 60% of the AU565 cells, and binds the same epitope as at least one reference antibody comprising the VH and VL regions of an antibody selected from the group consisting of 169, 091, 050, 084, 098, 025, 153, 129, 132, 127 and 159; preferably selected from antibodies 153, 132, 127, 129, 159 and 025.

In a preferred embodiment, the conjugated antibody binds to the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:49 and a VL region comprising the sequence of SEQ ID NO:53 (**159**).

In one embodiment, the conjugated antibody has a higher percentage of killed AU431 cells than trastuzumab and pertuzumab when determined as described in Example 18, preferably killing at least 50%, more preferably at least 70%, and binds the same epitope as at least one reference antibody comprising the VH and VL regions of an antibody selected from the group consisting of 025, 084, 091, 098, 129 and 153; preferably selected from antibodies 025, 091, 098, 129 and 153.

In a preferred embodiment, the conjugated antibody binds to the same epitope as a reference antibody comprising a VH region comprising the sequence of SEQ ID NO:56 and a VL region comprising the sequence of SEQ ID NO:60 (**098**).

In an additional or alternative embodiment, the antibody is internalized by tumor cells expressing HER2, such as AU565 cells, to a higher degree than trastuzumab and pertuzumab, preferably more than twice or three times the amount of internalized trastuzumab, preferably when determined according to Example 18, and binds to the same epitope as an antibody comprising VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:46 and a VL region comprising the sequence of SEQ ID NO:49 (**127**);
- b) a VH region comprising the sequence of SEQ ID NO:49 and a VL region comprising the sequence of SEQ ID NO:53 (**159**);
- c) a VH region comprising the sequence of SEQ ID NO:56 and a VL region comprising the sequence of SEQ ID NO:60 (**098**);
- d) a VH region comprising the sequence of SEQ ID NO:63 and a VL region comprising the sequence of SEQ ID NO:67 (**153**); and
- e) a VH region comprising the sequence of SEQ ID NO:70 and a VL region comprising the sequence of SEQ ID NO:74 (**132**).

Preferably, the antibody binds to the same epitope as an antibody comprising VH and VL regions selected from

- a) a VH region comprising the sequence of SEQ ID NO:46 and a VL region comprising the sequence of SEQ ID NO:49 (**127**) and
- b) a VH region comprising the sequence of SEQ ID NO:56 and a VL region comprising the sequence of SEQ ID NO:60 (**098**).

In a further embodiment, the antibody binds to Domain II or IV of HER2, preferably wherein the antibody does not significantly promote proliferation of HER2 expressing cells, and is more efficiently internalized, or is internalized to a higher degree, than trastuzumab or pertuzumab into HER2-expressing tumor cells, preferably when determined as described in the Examples, e.g. examples 16 and 19, respectively.

In a further embodiment the antibody enhanced HER2 downmodulation more than trastuzumab, e.g. the antibody enhanced HER2 downmodulation by more 30%, such as more than 40% or more than 50% when determined as described in example 22, preferably wherein the antibody binds to the same epitope as an antibody of cross-block group 3 of the present invention, e.g. an antibody binding to the same epitope as an antibody comprising VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:56 and a VL region comprising the sequence of SEQ ID NO:60 (**098**);
- b) a VH region comprising the sequence of SEQ ID NO:63 and a VL region comprising the sequence of SEQ ID NO:67 (**153**).

In another or alternative embodiment the antibody decreased tumour growth and improved survival in vivo more than trastuzumab, when determined as described in example 29, preferably wherein the antibody binds to the same epitope as an antibody of cross-block 1 or cross-block 2 of the present invention, e.g. an antibody binding to the same epitope as an antibody comprising VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:1 and a VL region comprising the sequence of SEQ ID NO:5 (**169**);
- b) a VH region comprising the sequence of SEQ ID NO:15 and a VL region comprising the sequence of SEQ ID NO:19 (**084**); and
- c) a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:32 (**091**).

In another or alternative embodiment the antibody decreased tumour growth and improved survival in vivo more than trastuzumab, when determined as described in example 30,

preferably wherein the antibody binds to the same epitope as an antibody of cross-block 2 or cross-block 3 of the present invention, e.g. an antibody binding to the same epitope as an antibody comprising VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:22 and a VL region comprising the sequence of SEQ ID NO:26 (**025**);
- b) a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:32 (**091**);
- c) a VH region comprising the sequence of SEQ ID NO:35 and a VL region comprising the sequence of SEQ ID NO:39 (**129**); and
- d) a VH region comprising the sequence of SEQ ID NO:63 and a VL region comprising the sequence of SEQ ID NO:67 (**153**).

More particularly, wherein the antibody binds to the same epitope as an antibody comprising VH and VL regions selected from the group consisting of:

- a) a VH region comprising the sequence of SEQ ID NO:22 and a VL region comprising the sequence of SEQ ID NO:26 (**025**); and
- b) a VH region comprising the sequence of SEQ ID NO:29 and a VL region comprising the sequence of SEQ ID NO:32 (**091**).

In one embodiment the antibody is a bispecific antibody.

In a further embodiment the antibody is a bispecific antibody which enhanced HER2 downmodulation, in particular more than their monospecific counterparts, e.g. the antibody enhanced HER2 downmodulation by more 20%, such as more than 30% or more than 40% when determined as described in example 22, preferably wherein the antibody binds to the same epitopes as bispecific antibody selected from the group consisting of IgG1-005-ITL x IgG1-169-K409R, IgG1-025-ITL x IgG1-005-K409R, IgG1-025-ITL x IgG1-153-K409R, IgG1-025-ITL x IgG1-169-K409R, IgG1-153-ITL x IgG1-005-K409R; and IgG1-153-ITL x IgG1-169-K409R.

In an additional or alternative embodiment, the bispecific antibody specifically binds HER2-expressing AU565 cells and inhibits ligand-induced proliferation of the cells when determined as described in Example 24, and binds the same epitopes as at least one bispecific antibody selected from the group consisting of: IgG1-005-ITL x IgG1-169-K409R, IgG1-025-ITL x IgG1-005-K409R, IgG1-025-ITL x IgG1-153-K409R, IgG1-025-ITL x IgG1-169-K409R, IgG1-153-ITL x IgG1-005-K409R; and IgG1-153-ITL x IgG1-169-K409R. In particular the bispecific antibody inhibits proliferation of the AU565 cells more than their

monospecific counterparts and is selected from the group consisting of IgG1-005-ITL x IgG1-169-K409R and IgG1-025-ITL x IgG1-005-K409R.

In an additional or alternative embodiment the bispecific antibody is a HER2 x CD3 bispecific antibody induce T cell mediated cytotoxicity of AU565 as described in example 25, and binds the same epitopes as at least one of the bispecific antibodies selected from the group consisting of: Duo huCLB-Q/153-Q, Duo huCLB-Q/B12-Q, Duo YTH12.5/153-Q and Duo YTH12.5/B12-Q (Duo indicating bispecific antibody).

#### Antibody formats

The present invention provides HER2 antibodies which efficiently bind to and internalize into HER2-expressing tumor cells, typically without significantly promoting ligand-independent proliferation of the cells. Depending on the desired functional properties for a particular use, particular antibodies can be selected from the set of antibodies provided in the present invention and/or their format can be adapted to change these properties, as described below.

The antibody of the invention can be of any isotype. The choice of isotype typically will be guided by the desired effector functions, such as ADCC induction. Exemplary isotypes are IgG1, IgG2, IgG3, and IgG4. Either of the human light chain constant regions, kappa or lambda, may be used. If desired, the class of a HER2 antibody of the present invention may be switched by known methods. For example, an antibody of the present invention that was originally IgM may be class switched to an IgG antibody of the present invention. Further, class switching techniques may be used to convert one IgG subclass to another, for instance from IgG1 to IgG2. Thus, the effector function of the antibodies of the present invention may be changed by isotype switching to, e.g., an IgG1, IgG2, IgG3, IgG4, IgD, IgA, IgE, or IgM antibody for various therapeutic uses. In one embodiment an antibody of the present invention is an IgG1 antibody, for instance an IgG1, $\kappa$ .

In a further embodiment, the antibody of the invention is glyco-engineered to reduce fucose and thus enhance ADCC, e.g. by addition of compounds to the culture media during antibody production as described in US2009317869 or as described in van Berkel *et al.* (2010) Biotechnol. Bioeng. 105:350 or by using FUT8 knockout cells, e.g. as described in Yamane-Ohnuki *et al.* (2004) Biotechnol. Bioeng 87:614. ADCC may alternatively be optimized using the method described by Umaña *et al.* (1999) Nature Biotech 17:176.

In a further embodiment, the antibody of the invention has been engineered to enhance complement activation, e.g. as described in Natsume *et al.* (2009) Cancer Sci. 100:2411.

In one embodiment, the antibody of the invention is a full-length antibody, preferably an IgG1 antibody, in particular an IgG1, $\kappa$  antibody. In another embodiment, the antibody of the invention is an antibody fragment or a single-chain antibody.

Antibody fragments may e.g. be obtained by fragmentation using conventional techniques, and the fragments screened for utility in the same manner as described herein for whole antibodies. For example, F(ab')<sub>2</sub> fragments may be generated by treating an antibody with pepsin. The resulting F(ab')<sub>2</sub> fragment may be treated to reduce disulfide bridges with a reducing agent, such as dithiothreitol, to produce Fab' fragments. Fab fragments may be obtained by treating an antibody with papain. A F(ab')<sub>2</sub> fragment may also be produced by binding Fab' fragments via a thioether bond or a disulfide bond. Antibody fragments may also be generated by expression of nucleic acids encoding such fragments in recombinant cells (see for instance Evans *et al.*, J. Immunol. Meth. 184, 123-38 (1995)). For example, a chimeric gene encoding a portion of an F(ab')<sub>2</sub> fragment could include DNA sequences encoding the C<sub>H</sub>1 domain and hinge region of the H chain, followed by a translational stop codon to yield such a truncated antibody fragment molecule.

As explained above, in one embodiment, the HER2 antibody of the invention is a bivalent antibody, i.e. an antibody capable of binding two antigens or epitopes on the same antigen.

In another embodiment, the HER2 antibody of the invention is a monovalent antibody.

In one embodiment, the antibody of the invention is a Fab fragment or a one-armed antibody, such as described in US20080063641 (Genentech) or other monovalent antibody, e.g. such as described in WO2007048037 (Amgen).

In a preferred embodiment, a monovalent antibody has a structure as described in WO2007059782 (Genmab) having a deletion of the hinge region. Accordingly, in one embodiment, the antibody is a monovalent antibody, wherein said HER2 antibody is constructed by a method comprising:

- i) providing a nucleic acid construct encoding the light chain of said monovalent antibody, said construct comprising a nucleotide sequence encoding the VL region of a selected antigen specific HER2 antibody and a nucleotide sequence encoding the constant CL region of an Ig, wherein said nucleotide sequence encoding the VL region of a selected antigen specific antibody and said nucleotide sequence encoding the CL region of an Ig are operably linked together, and wherein, in case of an IgG1 subtype, the nucleotide sequence encoding the CL region has been modified such that the CL region does not contain any amino acids capable of forming disulfide bonds or covalent bonds with other peptides

comprising an identical amino acid sequence of the CL region in the presence of polyclonal human IgG or when administered to an animal or human being;

- ii) providing a nucleic acid construct encoding the heavy chain of said monovalent antibody, said construct comprising a nucleotide sequence encoding the VH region of a selected antigen specific antibody and a nucleotide sequence encoding a constant CH region of a human Ig, wherein the nucleotide sequence encoding the CH region has been modified such that the region corresponding to the hinge region and, as required by the Ig subtype, other regions of the CH region, such as the CH3 region, does not comprise any amino acid residues which participate in the formation of disulphide bonds or covalent or stable non-covalent inter-heavy chain bonds with other peptides comprising an identical amino acid sequence of the CH region of the human Ig in the presence of polyclonal human IgG or when administered to an animal human being, wherein said nucleotide sequence encoding the VH region of a selected antigen specific antibody and said nucleotide sequence encoding the CH region of said Ig are operably linked together;
- iii) providing a cell expression system for producing said monovalent antibody;
- iv) producing said monovalent antibody by co-expressing the nucleic acid constructs of (i) and (ii) in cells of the cell expression system of (iii).

Similarly, in one embodiment, the HER2 antibody is a monovalent antibody, which comprises

- (i) a variable region of an antibody of the invention as described herein or an antigen binding part of the said region, and
- (ii) a C<sub>H</sub> region of an immunoglobulin or a fragment thereof comprising the C<sub>H</sub>2 and C<sub>H</sub>3 regions, wherein the C<sub>H</sub> region or fragment thereof has been modified such that the region corresponding to the hinge region and, if the immunoglobulin is not an IgG4 subtype, other regions of the C<sub>H</sub> region, such as the C<sub>H</sub>3 region, do not comprise any amino acid residues, which are capable of forming disulfide bonds with an identical C<sub>H</sub> region or other covalent or stable non-covalent inter-heavy chain bonds with an identical C<sub>H</sub> region in the presence of polyclonal human IgG.

In a further embodiment hereof, the heavy chain of the monovalent HER2 antibody has been modified such that the entire hinge has been deleted.

In another further embodiment, the immunoglobulin referred to in step ii) above is of the IgG4 subtype.

In another further embodiment, said monovalent antibody is of the IgG4 subtype, but the C<sub>H</sub>3 region has been modified so that one or more of the following amino acid substitutions have been made:

| Numbering of CH3 mutations |               |                                                                      |
|----------------------------|---------------|----------------------------------------------------------------------|
| KABAT*                     | EU index G4*  | Mutations                                                            |
| E378                       | E357          | E357A or E357T or E357V or E357I                                     |
| S387                       | S364          | S364R or S364K                                                       |
| T389                       | T366          | T366A or T366R or T366K or T366N                                     |
| L391                       | L368          | L368A or L368V or L368E or L368G or L368S or L368T                   |
| D427                       | D399          | D399A or D399T or D399S                                              |
| F436                       | F405          | F405A or F405L or F405T or F405D or F405R or F405Q or F405K or F405Y |
| Y438                       | Y407          | Y407A or Y407E or Y407Q or Y407K or Y407F                            |
| F436 and Y438              | F405 and Y407 | (F405T and Y407E) or (F405D and Y407E)                               |
|                            |               | (D399S and Y407Q) or (D399S and Y407K) or (D399S                     |
| D427 and Y438              | D399 and Y407 | and Y407E)                                                           |

\*KABAT indicates amino acid numbering according to Kabat (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991). EU index indicates amino acid numbering according to EU index as outlined in Kabat *et al.*, (*supra*).

In another further embodiment, the sequence of said monovalent antibody has been modified so that it does not comprise any acceptor sites for N-linked glycosylation.

HER2 antibodies of the invention also include single chain antibodies. Single chain antibodies are peptides in which the heavy and light chain Fv regions are connected. In one embodiment, the present invention provides a single-chain Fv (scFv) wherein the heavy and light chains in the Fv of a HER2 antibody of the present invention are joined with a flexible peptide linker (typically of about 10, 12, 15 or more amino acid residues) in a single peptide chain. Methods of producing such antibodies are described in for instance US 4,946,778, Pluckthun in *The Pharmacology of Monoclonal Antibodies*, vol. 113, Rosenberg and Moore eds. Springer-Verlag, New York, pp. 269-315 (1994), Bird *et al.*, *Science* 242, 423-426 (1988), Huston *et al.*, *PNAS USA* 85, 5879-5883 (1988) and McCafferty *et al.*, *Nature* 348, 552-554 (1990). The single chain antibody may be monovalent, if only a single V<sub>H</sub> and V<sub>L</sub> are used, bivalent, if two V<sub>H</sub> and V<sub>L</sub> are used, or polyvalent, if more than two V<sub>H</sub> and V<sub>L</sub> are used.

In one embodiment, the HER2 antibody of the invention is an effector-function-deficient antibody. In one embodiment, the effector-function-deficient HER2 antibody is a human stabilized IgG4 antibody, which has been modified to prevent Fab-arm exchange (van der Neut Kolfschoten *et al.* (2007) *Science* 317(5844):1554-7). Examples of suitable

human stabilized IgG4 antibodies are antibodies, wherein arginine at position 409 in a heavy chain constant region of human IgG4, which is indicated in the EU index as in Kabat *et al.*, is substituted with lysine, threonine, methionine, or leucine, preferably lysine (described in WO2006033386 (Kirin)) and/or wherein the hinge region has been modified to comprise a Cys-Pro-Pro-Cys sequence.

In one embodiment, the stabilized IgG4 HER2 antibody is an IgG4 antibody comprising a heavy chain and a light chain, wherein said heavy chain comprises a human IgG4 constant region having a residue selected from the group consisting of: Lys, Ala, Thr, Met and Leu at the position corresponding to 409 and/or a residue selected from the group consisting of: Ala, Val, Gly, Ile and Leu at the position corresponding to 405, and wherein said antibody optionally comprises one or more further substitutions, deletions and/or insertions, but does not comprise a Cys-Pro-Pro-Cys sequence in the hinge region. Preferably, said antibody comprises a Lys or Ala residue at the position corresponding to 409 or the CH3 region of the antibody has been replaced by the CH3 region of human IgG1, of human IgG2 or of human IgG3. See also WO2008145142 (Genmab).

In an even further embodiment, the stabilized IgG4 HER2 antibody is an IgG4 antibody comprising a heavy chain and a light chain, wherein said heavy chain comprises a human IgG4 constant region having a residue selected from the group consisting of: Lys, Ala, Thr, Met and Leu at the position corresponding to 409 and/or a residue selected from the group consisting of: Ala, Val, Gly, Ile and Leu at the position corresponding to 405, and wherein said antibody optionally comprises one or more further substitutions, deletions and/or insertions and wherein said antibody comprises a Cys-Pro-Pro-Cys sequence in the hinge region. Preferably, said antibody comprises a Lys or Ala residue at the position corresponding to 409 or the CH3 region of the antibody has been replaced by the CH3 region of human IgG1, of human IgG2 or of human IgG3.

In a further embodiment, the effector-function-deficient HER2 antibody is an antibody of a non-IgG4 type, e.g. IgG1, IgG2 or IgG3 which has been mutated such that the ability to mediate effector functions, such as ADCC, has been reduced or even eliminated. Such mutations have e.g. been described in Dall'Acqua WF *et al.*, J Immunol. 177(2):1129-1138 (2006) and Hezareh M, J Virol. ;75(24):12161-12168 (2001).

#### Conjugates

In a further embodiment, the present invention provides a HER2 antibody or an HER2 bispecific antibody linked or conjugated to a therapeutic moiety, such as a cytotoxin, a chemotherapeutic drug, a cytokine, an immunosuppressant, or a radioisotope. Such

conjugates are referred to herein as "immunoconjugates". Immunoconjugates which include one or more cytotoxins are referred to as "immunotoxins".

A cytotoxin or cytotoxic agent includes any agent that is detrimental to (e.g., kills) cells. Suitable therapeutic agents for forming immunoconjugates of the present invention include taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, maytansine or an analog or derivative thereof, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin,; calicheamicin or analogs or derivatives thereof; antimetabolites (such as methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, fludarabin, 5-fluorouracil, decarbazine, hydroxyurea, asparaginase, gemcitabine, cladribine), alkylating agents (such as mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, dacarbazine (DTIC), procarbazine, mitomycin C, cisplatin and other platinum derivatives, such as carboplatin; as well as duocarmycin A, duocarmycin SA, CC-1065 (a.k.a. rachemycin), or analogs or derivatives of CC-1065), antibiotics (such as dactinomycin (formerly actinomycin), bleomycin, daunorubicin (formerly daunomycin), doxorubicin, idarubicin, mithramycin, mitomycin, mitoxantrone, plicamycin, anthramycin (AMC)), anti-mitotic agents (e.g., tubulin-inhibitors) such as monomethyl auristatin E, monomethyl auristatin F, or other analogs or derivatives of dolastatin 10; diphtheria toxin and related molecules (such as diphtheria A chain and active fragments thereof and hybrid molecules); ricin toxin (such as ricin A or a deglycosylated ricin A chain toxin), cholera toxin, a Shiga-like toxin (SLT-I, SLT-II, SLT-IIV), LT toxin, C3 toxin, Shiga toxin, pertussis toxin, tetanus toxin, soybean Bowman-Birk protease inhibitor, Pseudomonas exotoxin, alorin, saporin, modeccin, gelatin, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana proteins (PAPI, PAPII, and PAP-S), momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, and enomycin toxins. Other suitable conjugated molecules include antimicrobial/lytic peptides such as CLIP, Magainin 2, mellitin, Cecropin, and P18; ribonuclease (RNase), DNase I, Staphylococcal enterotoxin-A, pokeweed antiviral protein, diphtherin toxin, and Pseudomonas endotoxin. See, for example, Pastan *et al.*, Cell 47, 641 (1986) and Goldenberg, Calif. A Cancer Journal for Clinicians 44, 43 (1994). Therapeutic agents that may be administered in combination with a HER2 antibody of the present invention as described elsewhere herein, such as, e.g., anti-cancer cytokines or chemokines, are also candidates for therapeutic moieties useful for conjugation to an antibody of the present invention.

In one embodiment, a HER2 antibody of the invention comprises a conjugated nucleic acid or nucleic acid-associated molecule. In one such embodiment, the conjugated nucleic acid is a cytotoxic ribonuclease, an antisense nucleic acid, an inhibitory RNA molecule (e.g., a siRNA molecule) or an immunostimulatory nucleic acid (e.g., an immunostimulatory CpG motif-containing DNA molecule). In another embodiment, a HER2 antibody of the invention is conjugated to an aptamer or a ribozyme.

In one embodiment, HER2 antibodies comprising one or more radiolabeled amino acids are provided. A radiolabeled HER2 antibody may be used for both diagnostic and therapeutic purposes (conjugation to radiolabeled molecules is another possible feature). Non-limiting examples of labels for polypeptides include  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{99}\text{Tc}$ , and  $^{125}\text{I}$ ,  $^{131}\text{I}$ , and  $^{186}\text{Re}$ .

In one embodiment, the antibody is conjugated to a radioisotope or to a radioisotope-containing chelate. For example, the antibody can be conjugated to a chelator linker, e.g. DOTA, DTPA or tiuxetan, which allows for the antibody to be complexed with a radioisotope. The antibody may also or alternatively comprise or be conjugated to one or more radiolabeled amino acids or other radiolabeled molecule. A radiolabeled CD74 Ab may be used for both diagnostic and therapeutic purposes. Non-limiting examples of radioisotopes include  $^3\text{H}$ ,  $^{14}\text{C}$ ,  $^{15}\text{N}$ ,  $^{35}\text{S}$ ,  $^{90}\text{Y}$ ,  $^{99}\text{Tc}$ ,  $^{125}\text{I}$ ,  $^{111}\text{In}$ ,  $^{131}\text{I}$ ,  $^{186}\text{Re}$ ,  $^{213}\text{Bs}$ ,  $^{225}\text{Ac}$  and  $^{227}\text{Th}$ .

HER2 antibodies may also be chemically modified by covalent conjugation to a polymer to for instance increase their circulating half-life. Exemplary polymers, and methods to attach them to peptides, are illustrated in for instance US 4,766,106, US 4,179,337, US 4,495,285 and US 4,609,546. Additional polymers include polyoxyethylated polyols and polyethylene glycol (PEG) (e.g., a PEG with a molecular weight of between about 1,000 and about 40,000, such as between about 2,000 and about 20,000).

Any method known in the art for conjugating the HER2 antibody to the conjugated molecule(s), such as those described above, may be employed, including the methods described by Hunter *et al.*, *Nature* **144**, 945 (1962), David *et al.*, *Biochemistry* **13**, 1014 (1974), Pain *et al.*, *J. Immunol. Meth.* **40**, 219 (1981) and Nygren, *J. Histochem. and Cytochem.* **30**, 407 (1982). Such antibodies may be produced by chemically conjugating the other moiety to the N-terminal side or C-terminal side of the HER2 antibody or fragment thereof (e.g., a HER2 antibody H or L chain) (see, e.g., *Antibody Engineering Handbook*, edited by Osamu Kanemitsu, published by Chijin Shokan (1994)). Such conjugated antibody derivatives may also be generated by conjugation at internal residues or sugars, where appropriate.

The agents may be coupled either directly or indirectly to a HER2 antibody of the present invention. One example of indirect coupling of a second agent is coupling via a

spacer moiety to cysteine or lysine residues in the antibody. In one embodiment, a HER2 antibody is conjugated to a prodrug molecule that can be activated *in vivo* to a therapeutic drug via a spacer or linker. After administration, the spacers or linkers are cleaved by tumor-cell associated enzymes or other tumor-specific conditions, by which the active drug is formed. Examples of such prodrug technologies and linkers are described in WO02083180, WO2004043493, WO2007018431, WO2007089149, and WO2009017394 by Syntarga BV, *et al.* Suitable antibody-prodrug technology and duocarmycin analogs can also be found in U.S. Patent No. 6,989,452 (Medarex).

In one embodiment, the HER2 antibody of the present invention is attached to a chelator linker, e.g. tiuxetan, which allows for the antibody to be conjugated to a radioisotope.

#### Bispecific antibodies

In a further aspect, the invention relates to a bispecific molecule comprising a first antigen binding site from a HER2 antibody of the invention as described herein above and a second antigen binding site with a different binding specificity, such as a binding specificity for a human effector cell, a human Fc receptor, a T cell receptor, a B cell receptor or a binding specificity for a non-overlapping epitope of HER2, i.e. a bispecific antibody wherein the first and second antigen binding sites do not cross-block each other for binding to HER2, e.g. when tested as described in Example 14.

Exemplary bispecific antibody molecules of the invention comprise (i) two antibodies, one with a specificity to HER2 and another to a second target that are conjugated together, (ii) a single antibody that has one chain or arm specific to HER2 and a second chain or arm specific to a second molecule, (iii) a single chain antibody that has specificity to HER2 and a second molecule, e.g., via two scFvs linked in tandem by an extra peptide linker; (iv) a dual-variable-domain antibody (DVD-Ig), where each light chain and heavy chain contains two variable domains in tandem through a short peptide linkage (Wu *et al.*, Generation and Characterization of a Dual Variable Domain Immunoglobulin (DVD-Ig™) Molecule, In: Antibody Engineering, Springer Berlin Heidelberg (2010)); (v) a chemically-linked bispecific (Fab')<sub>2</sub> fragment; (vi) a Tandab, which is a fusion of two single chain diabodies resulting in a tetravalent bispecific antibody that has two binding sites for each of the target antigens; (vii) a flexibody, which is a combination of scFvs with a diabody resulting in a multivalent molecule; (viii) a so called "dock and lock" molecule, based on the "dimerization and docking domain" in Protein Kinase A, which, when applied to Fabs, can yield a trivalent bispecific binding protein consisting of two identical Fab fragments linked to a different Fab fragment; (ix) a so-called Scorpion molecule, comprising, e.g., two scFvs fused to both termini of a human Fc-region; and (x) a diabody. In one embodiment, the bispecific

antibody of the present invention is a diabody, a cross-body, or a bispecific obtained via a controlled Fab arm exchange as those described in the present invention.

Examples of platforms useful for preparing bispecific antibodies include but are not limited to BiTE (Micromet), DART (MacroGenics), Fcab and Mab<sup>2</sup> (F-star) , Fc-engineered IgG1 (Xencor) or DuoBody (based on Fab arm exchange, Genmab, this application).

Examples of different classes of bispecific antibodies include but are not limited to

- asymmetric IgG-like molecules, wherein the one side of the molecule contains the Fab region or part of the Fab region of at least one antibody, and the other side of the molecule contains the Fab region or parts of the Fab region of at least one other antibody; in this class, asymmetry in the Fc region could also be present, and be used for specific linkage of the two parts of the molecule;
- symmetric IgG-like molecules, wherein the two sides of the molecule each contain the Fab region or part of the Fab region of at least two different antibodies;
- IgG fusion molecules, wherein full length IgG antibodies are fused to extra Fab regions or parts of Fab regions;
- Fc fusion molecules, wherein single chain Fv molecules or stabilized diabodies are fused to Fc $\gamma$  regions or parts thereof,;
- Fab fusion molecules, wherein different Fab-fragments are fused together;
- ScFv-and diabody-based molecules wherein different single chain Fv molecules or different diabodies are fused to each other or to another protein or carrier molecule.

Examples of asymmetric IgG-like molecules include but are not limited to the Triomab/Quadroma (Trion Pharma/Fresenius Biotech), the Knobs-into-Holes (Genentech), CrossMAbs (Roche) and the electrostatically-matched (Amgen), the LUZ-Y (Genentech), the Strand Exchange Engineered Domain body (EMD Serono), the Biclonic (Merus) and the DuoBody (Genmab A/S).

Example of symmetric IgG-like molecules include but are not limited to Dual Targeting (DT)-Ig (GSK/Domantis), Two-in-one Antibody (Genentech), Cross-linked Mabs (Karmanos Cancer Center), mAb<sup>2</sup> (F-Star) and CovX-body (CovX/Pfizer).

Examples of IgG fusion molecules include but are not limited to Dual Variable Domain (DVD)-Ig (Abbott), IgG-like Bispecific (ImClone/Eli Lilly), Ts2Ab (MedImmune/AZ) and BsAb (Zymogenetics), HERCULES (Biogen Idec) and TvAb (Roche).

Examples of Fc fusion molecules include but are not limited to ScFv/Fc Fusions (Academic Institution), SCORPION (Emergent BioSolutions/Trubion, Zymogenetics/BMS), Dual Affinity Retargeting Technology (Fc-DART) (MacroGenics) and Dual(ScFv)<sub>2</sub>-Fab (National Research Center for Antibody Medicine – China).

Examples of class V bispecific antibodies include but are not limited to F(ab)<sub>2</sub> (Medarex/AMGEN), Dual-Action or Bis-Fab (Genentech), Dock-and-Lock (DNL) (ImmunoMedics), Bivalent Bispecific (Biotechnol) and Fab-Fv (UCB-Celltech). Examples of ScFv-and diabody-based molecules include but are not limited to Bispecific T Cell Engager (BiTE) (Micromet9, Tandem Diabody (Tandab) (Affimed), Dual Affinity Retargeting Technology (DART) (MacroGenics), Single-chain Diabody (Academic), TCR-like Antibodies (AIT, ReceptorLogics), Human Serum Albumin ScFv Fusion (Merrimack) and COMBODY (Epigen Biotech).

In one embodiment, the second molecule is a cancer antigen/tumor-associated antigen such as carcinoembryonic antigen (CEA), prostate specific antigen (PSA), RAGE (renal antigen),  $\alpha$ -fetoprotein, CAMEL (CTL-recognized antigen on melanoma), CT antigens (such as MAGE-B5, -B6, -C2, -C3, and D; Mage-12; CT10; NY-ESO-1, SSX-2, GAGE, BAGE, MAGE, and SAGE), mucin antigens (e.g., MUC1, mucin-CA125, etc.), ganglioside antigens, tyrosinase, gp75, c-Met, C-myc, Mart1, MelanA, MUM-1, MUM-2, MUM-3, HLA-B7, Ep-CAM or a cancer-associated integrin, such as  $\alpha 5 \beta 3$  integrin. In another embodiment, the second molecule is a T cell and/or NK cell antigen, such as CD3 or CD16. In another embodiment, the second molecule is an angiogenic factor or other cancer-associated growth factor, such as a vascular endothelial growth factor, a fibroblast growth factor, epidermal growth factor, angiogenin or a receptor of any of these, particularly receptors associated with cancer progression (for instance another one of the HER receptors; HER1, HER3, or HER4). In one embodiment, the second antigen-binding site binds a different, preferably non-blocking, site on HER2 than the one bound by the antibody of the invention. For example, the second molecule may be derived from, or cross-block HER2-binding of, trastuzumab, pertuzumab, F5, or C1.

Methods of preparing bispecific antibodies include those described in WO 2008119353 (Genmab) and reported van der Neut-Kolfschoten et al. (Science. 2007 Sep 14;317(5844):1554-7) and it may for example be performed as described in example 20 of the present invention.

#### Nucleic acid sequences, vectors and host cells

In a further aspect, the invention relates to nucleic acid sequences, such as DNA sequences, encoding heavy and light chains of an antibody of the invention.

In one embodiment, the nucleic acid sequence encodes an amino acid sequence selected from the group consisting of: SEQ ID NO: 1, 5, 8, 12, 15, 19, 22, 26, 29, 32, 35, 39, 42, 46, 49, 53, 56, 60,, 63, 67, 70, 74, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108,

109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125 and 126.

In another particular embodiment, the nucleic acid sequence encodes a VH amino acid sequence selected from the group consisting of: SEQ ID NO: 1, 8, 15, 22, 29, 35, 42, 49, 56, 63, 70, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, and 125.

In another particular embodiment, the nucleic acid sequence encodes a VL amino acid sequence selected from the group consisting of: SEQ ID NO: 5, 12, 19, 26, 32, 39, 46, 53, 60, 67, 74, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, and 126.

In an even further aspect, the invention relates to an expression vector, or a set of expression vectors, encoding an antibody of the invention. The heavy and light chain of the antibody may be encoded by the same vector or by different vector.

Such expression vectors may be used for recombinant production of antibodies of the invention.

In one embodiment, the expression vector of the invention comprises a nucleotide sequence encoding one or more of the amino acid sequences selected from the group consisting of: SEQ ID NO: 1, 5, 8, 12, 15, 19, 22, 26, 29, 32, 35, 39, 42, 46, 49, 53, 56, 60,, 63, 67, 70, 74, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, 120, 121, 122, 123, 124, 125 and 126.

In another particular embodiment, the expression vector of the invention comprises a nucleotide sequence encoding one or more of the VH amino acid sequences selected from the group consisting of: SEQ ID NO: 1, 8, 15, 22, 29, 35, 42, 49, 56, 63, 70, 77, 79, 81, 83, 85, 87, 89, 91, 93, 95, 97, 99, 101, 103, 105, 107, 109, 111, 113, 115, 117, 119, 121, 123, and 125.

In another particular embodiment, the expression vector of the invention comprises a nucleotide sequence encoding one or more of the VL amino acid sequences selected from the group consisting of: SEQ ID NO: 5, 12, 19, 26, 32, 39, 46, 53, 60, 67, 74, 78, 80, 82, 84, 86, 88, 90, 92, 94, 96, 98, 100, 102, 104, 106, 108, 110, 112, 114, 116, 118, 120, 122, 124, and 126.

In a further embodiment, the expression vector further comprises a nucleotide sequence encoding the constant region of a light chain, a heavy chain or both light and heavy chains of an antibody, e.g. a human antibody.

An expression vector in the context of the present invention may be any suitable vector, including chromosomal, non-chromosomal, and synthetic nucleic acid vectors (a

nucleic acid sequence comprising a suitable set of expression control elements). Examples of such vectors include derivatives of SV40, bacterial plasmids, phage DNA, baculovirus, yeast plasmids, vectors derived from combinations of plasmids and phage DNA, and viral nucleic acid (RNA or DNA) vectors. In one embodiment, a HER2 antibody-encoding nucleic acid is comprised in a naked DNA or RNA vector, including, for example, a linear expression element (as described in for instance Sykes and Johnston, *Nat Biotech* 17, 355-59 (1997)), a compacted nucleic acid vector (as described in for instance US 6,077, 835 and/or WO 00/70087), a plasmid vector such as pBR322, pUC 19/18, or pUC 118/119, a "midge" minimally-sized nucleic acid vector (as described in for instance Schakowski et al., *Mol Ther* 3, 793-800 (2001)), or as a precipitated nucleic acid vector construct, such as a CaP04-precipitated construct (as described in for instance WO 00/46147, Benvenisty and Reshef, *PNAS USA* 83, 9551-55 (1986), Wigler et al., *Cell* 14, 725 (1978), and Coraro and Pearson, *Somatic Cell Genetics* 7, 603 (1981)). Such nucleic acid vectors and the usage thereof are well known in the art (see for instance US 5,589,466 and US 5,973,972).

Exemplary expression vectors for the antibodies of the invention are also described in Examples 2 and 3.

In one embodiment, the vector is suitable for expression of the HER2 antibody in a bacterial cell. Examples of such vectors include expression vectors such as BlueScript™ (Stratagene), pIN™ vectors (Van Heeke & Schuster, *J Biol Chem* 264, 5503-5509 (1989), pET™ vectors (Novagen, Madison WI) and the like).

An expression vector may also or alternatively be a vector suitable for expression in a yeast system. Any vector suitable for expression in a yeast system may be employed. Suitable vectors include, for example, vectors comprising constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH (reviewed in: F. Ausubel *et al.*, ed. *Current Protocols in Molecular Biology*, Greene Publishing and Wiley InterScience New York (1987), and Grant *et al.*, *Methods in Enzymol* 153, 516-544 (1987)).

An expression vector may also or alternatively be a vector suitable for expression in mammalian cells, e.g. a vector comprising glutamine synthetase as a selectable marker, such as the vectors described in Bebbington (1992) *Biotechnology (NY)* 10:169-175.

A nucleic acid and/or vector may also comprises a nucleic acid sequence encoding a secretion/localization sequence, which can target a polypeptide, such as a nascent polypeptide chain, to the periplasmic space or into cell culture media. Such sequences are known in the art, and include secretion leader or signal peptides.

In an expression vector of the invention, HER2 antibody-encoding nucleic acids may comprise or be associated with any suitable promoter, enhancer, and other expression-facilitating elements. Examples of such elements include strong expression promoters (e. g.,

human CMV IE promoter/enhancer as well as RSV, SV40, SL3-3, MMTV, and HIV LTR promoters), effective poly (A) termination sequences, an origin of replication for plasmid product in E. coli, an antibiotic resistance gene as selectable marker, and/or a convenient cloning site (e.g., a polylinker). Nucleic acids may also comprise an inducible promoter as opposed to a constitutive promoter such as CMV IE.

In one embodiment, the HER2 antibody-encoding expression vector may be positioned in and/or delivered to the host cell or host animal via a viral vector.

In an even further aspect, the invention relates to a recombinant eukaryotic or prokaryotic host cell, such as a transfectoma, which produces an antibody of the invention as defined herein. Examples of host cells include yeast, bacterial, and mammalian cells, such as CHO or HEK cells. For example, in one embodiment, the present invention provides a cell comprising a nucleic acid stably integrated into the cellular genome that comprises a sequence coding for expression of a HER2 antibody of the present invention. In another embodiment, the present invention provides a cell comprising a non-integrated nucleic acid, such as a plasmid, cosmid, phagemid, or linear expression element, which comprises a sequence coding for expression of a HER2 antibody of the invention.

In a further aspect, the invention relates to a hybridoma which produces an antibody of the invention as defined herein. In an even further aspect, the invention relates to a transgenic non-human animal or plant comprising nucleic acids encoding a human heavy chain and a human light chain, wherein the animal or plant produces an antibody of the invention of the invention.

In a further aspect, the invention relates to a method for producing a HER2 antibody of the invention, said method comprising the steps of  
a) culturing a hybridoma or a host cell of the invention as described herein above, and  
b) purifying the antibody of the invention from the culture media.

#### Compositions

In a further main aspect, the invention relates to a pharmaceutical composition comprising:

- a HER2 antibody as defined herein, and
- a pharmaceutically-acceptable carrier.

The pharmaceutical composition of the present invention may contain one antibody of the present invention or a combination of different antibodies of the present invention.

The pharmaceutical compositions may be formulated in accordance with conventional techniques such as those disclosed in Remington: The Science and Practice of Pharmacy, 19th Edition, Gennaro, Ed., Mack Publishing Co., Easton, PA, 1995. A pharmaceutical

composition of the present invention may e.g. include diluents, fillers, salts, buffers, detergents (e. g., a nonionic detergent, such as Tween™-20 or Tween™-80), stabilizers (e. g., sugars or protein-free amino acids), preservatives, tissue fixatives, solubilizers, and/or other materials suitable for inclusion in a pharmaceutical composition.

Pharmaceutically acceptable carriers include any and all suitable solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonicity agents, antioxidants and absorption delaying agents, and the like that are physiologically compatible with a compound of the present invention. Examples of suitable aqueous and nonaqueous carriers which may be employed in the pharmaceutical compositions of the present invention include water, saline, phosphate buffered saline, ethanol, dextrose, polyols (such as glycerol, propylene glycol, polyethylene glycol, and the like), and suitable mixtures thereof, vegetable oils, carboxymethyl cellulose colloidal solutions, tragacanth gum and injectable organic esters, such as ethyl oleate, and/or various buffers. Pharmaceutically acceptable carriers include sterile aqueous solutions or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. Proper fluidity may be maintained, for example, by the use of coating materials, such as lecithin, by the maintenance of the required particle size in the case of dispersions, and by the use of surfactants.

Pharmaceutical compositions of the present invention may also comprise pharmaceutically acceptable antioxidants for instance (1) water soluble antioxidants, such as ascorbic acid, cysteine hydrochloride, sodium bisulfate, sodium metabisulfite, sodium sulfite and the like; (2) oil-soluble antioxidants, such as ascorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, lecithin, propyl gallate, alpha-tocopherol, and the like; and (3) metal chelating agents, such as citric acid, ethylenediamine tetraacetic acid (EDTA), sorbitol, tartaric acid, phosphoric acid, and the like.

Pharmaceutical compositions of the present invention may also comprise isotonicity agents, such as sugars, polyalcohols, such as mannitol, sorbitol, glycerol or sodium chloride in the compositions.

The pharmaceutical compositions of the present invention may also contain one or more adjuvants appropriate for the chosen route of administration such as preservatives, wetting agents, emulsifying agents, dispersing agents, preservatives or buffers, which may enhance the shelf life or effectiveness of the pharmaceutical composition. The compounds of the present invention may be prepared with carriers that will protect the compound against rapid release, such as a controlled release formulation, including implants, transdermal patches, and microencapsulated delivery systems. Such carriers may include gelatin, glyceryl monostearate, glyceryl distearate, biodegradable, biocompatible polymers such as

ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid alone or with a wax, or other materials well known in the art. Methods for the preparation of such formulations are generally known to those skilled in the art.

Sterile injectable solutions may be prepared by incorporating the active compound in the required amount in an appropriate solvent with one or a combination of ingredients e.g. as enumerated above, as required, followed by sterilization microfiltration. Generally, dispersions are prepared by incorporating the active compound into a sterile vehicle that contains a basic dispersion medium and the required other ingredients e.g. from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, examples of methods of preparation are vacuum drying and freeze-drying (lyophilization) that yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The actual dosage levels of the active ingredients in the pharmaceutical compositions may be varied so as to obtain an amount of the active ingredient which is effective to achieve the desired therapeutic response for a particular patient, composition, and mode of administration, without being toxic to the patient. The selected dosage level will depend upon a variety of pharmacokinetic factors including the activity of the particular compositions of the present invention employed, or the amide thereof, the route of administration, the time of administration, the rate of excretion of the particular compound being employed, the duration of the treatment, other drugs, compounds and/or materials used in combination with the particular compositions employed, the age, sex, weight, condition, general health and prior medical history of the patient being treated, and like factors well known in the medical arts.

The pharmaceutical composition may be administered by any suitable route and mode. In one embodiment, a pharmaceutical composition of the present invention is administered parenterally. "Administered parenterally" as used herein means modes of administration other than enteral and topical administration, usually by injection, and include epidermal, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, intratendinous, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, intracranial, intrathoracic, epidural and intrasternal injection and infusion.

In one embodiment that pharmaceutical composition is administered by intravenous or subcutaneous injection or infusion.

#### Uses

In a further main aspect, the invention relates to a HER2 antibody of the invention for use as a medicament.

The HER2 antibodies of the invention may be used for a number of purposes. In particular, the antibodies of the invention may be used for the treatment of various forms of cancer, including metastatic cancer and refractory cancer.

In one embodiment, the HER2 antibodies of the invention are used for the treatment of breast cancer, including primary, metastatic, and refractory breast cancer.

In one embodiment, the HER2 antibodies of the invention are used for the treatment of a form of cancer selected from the group consisting of prostate cancer, non-small cell lung cancer, bladder cancer, ovarian cancer, gastric cancer, colorectal cancer, esophageal cancer, squamous cell carcinoma of the head & neck, cervical cancer, pancreatic cancer, testis cancer, malignant melanoma and a soft-tissue cancer (e.g. synovial sarcoma).

Similarly, the invention relates to a method for killing a tumor cell expressing HER2, comprising administration, to an individual in need thereof, of an effective amount of an antibody of the invention, such as an antibody drug-conjugate (ADC).

In one embodiment, said tumor cell is involved in a form of cancer selected from the group consisting of: breast cancer, prostate cancer, non-small cell lung cancer, bladder cancer, ovarian cancer, gastric cancer, colorectal cancer, esophageal cancer and squamous cell carcinoma of the head & neck, cervical cancer, pancreatic cancer, testis cancer, malignant melanoma, and a soft-tissue cancer (e.g., synovial sarcoma).

In one embodiment, the tumor cell is one that co-expresses HER2 and at least one other member of the EGFR family, preferably EGFR, HER3, or both of EGFR and HER3, and is a tumor cell involved in breast cancer, colorectal cancer, endometrial/cervical cancer, lung cancer, malignant melanoma, ovarian cancer, pancreatic cancer, prostate cancer, testis cancer, a soft-tissue tumor (e.g., synovial sarcoma), or bladder cancer.

In one aspect, the invention relates to a method for treating cancer in a subject, comprising selecting a subject suffering from a cancer comprising tumor cells co-expressing HER2 and EGFR and/or HER3, and administering to the subject an antibody of the invention, optionally in the form of an antibody conjugated to a cytotoxic agent or drug. In one embodiment, the subject suffers from a cancer selected from the group consisting of breast cancer, colorectal cancer, endometrial/cervical cancer, lung cancer, malignant melanoma, ovarian cancer, pancreatic cancer, prostate cancer, testis cancer, a soft-tissue tumor (e.g., synovial sarcoma), or bladder cancer.

Also, the invention relates to the use of a monoclonal antibody that binds to human HER2 for the preparation of a medicament for the treatment of cancer, such as one of the specific cancer indications mentioned above.

The invention further relates to a monoclonal antibody for use in the treatment of cancer, such as one of the cancer indications mentioned above.

In a further embodiment of the methods of treatment of the present invention, the efficacy of the treatment is being monitored during the therapy, e.g. at predefined points in time, by determining tumor burden or HER2 expression levels on the relevant tumor cells.

*Dosage regimens in the above methods of treatment and uses are adjusted to provide the optimum desired response (e.g., a therapeutic response). For example, a single bolus may be administered, several divided doses may be administered over time or the dose may be proportionally reduced or increased as indicated by the exigencies of the therapeutic situation. Parenteral compositions may be formulated in dosage unit form for ease of administration and uniformity of dosage.*

The efficient dosages and the dosage regimens for the HER2 antibodies depend on the disease or condition to be treated and may be determined by the persons skilled in the art. An exemplary, non-limiting range for a therapeutically effective amount of a compound of the present invention is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, about 3, about 5, or about 8 mg/kg.

A physician or veterinarian having ordinary skill in the art may readily determine and prescribe the effective amount of the pharmaceutical composition required. For example, the physician or veterinarian could start doses of the HER2 antibody employed in the pharmaceutical composition at levels lower than that required in order to achieve the desired therapeutic effect and gradually increase the dosage until the desired effect is achieved. In general, a suitable daily dose of a composition of the present invention will be that amount of the compound which is the lowest dose effective to produce a therapeutic effect. Administration may e.g. be parenteral, such as intravenous, intramuscular or subcutaneous. In one embodiment, the HER2 antibodies may be administered by infusion in a weekly dosage of from 10 to 500 mg/m<sup>2</sup>, such as of from 200 to 400 mg/m<sup>2</sup>. Such administration may be repeated, e.g., 1 to 8 times, such as 3 to 5 times. The administration may be performed by continuous infusion over a period of from 2 to 24 hours, such as of from 2 to 12 hours. In one embodiment, the HER2 antibodies may be administered by slow continuous infusion over a long period, such as more than 24 hours, in order to reduce toxic side effects.

In one embodiment the HER2 antibodies may be administered in a weekly dosage of from 250 mg to 2000 mg, such as for example 300 mg, 500 mg, 700 mg, 1000 mg, 1500 mg or 2000 mg, for up to 8 times, such as from 4 to 6 times when given once a week. Such regimen may be repeated one or more times as necessary, for example, after 6 months or

12 months. The dosage may be determined or adjusted by measuring the amount of compound of the present invention in the blood upon administration by for instance taking out a biological sample and using anti-idiotypic antibodies which target the antigen binding region of the HER2 antibodies of the present invention.

The efficient dosages and the dosage regimens for the bispecific antibodies depend on the disease or condition to be treated and may be determined by the persons skilled in the art. An exemplary, non-limiting range for a therapeutically effective amount of a bispecific antibody of the present invention is about 0.1-100 mg/kg, such as about 0.1-50 mg/kg, for example about 0.1-20 mg/kg, such as about 0.1-10 mg/kg, for instance about 0.5, about such as 0.3, about 1, about 3, about 5, or about 8 mg/kg.

In one embodiment, the HER2 antibodies may be administered by maintenance therapy, such as, e.g., once a week for a period of 6 months or more.

A HER2 antibody may also be administered prophylactically in order to reduce the risk of developing cancer, delay the onset of the occurrence of an event in cancer progression, and/or reduce the risk of recurrence when a cancer is in remission.

HER2 antibodies may also be administered in combination therapy, i.e., combined with other therapeutic agents relevant for the disease or condition to be treated. Accordingly, in one embodiment, the antibody-containing medicament is for combination with one or more further therapeutic agent, such as a cytotoxic, chemotherapeutic or anti-angiogenic agent.

Such combined administration may be simultaneous, separate or sequential. For simultaneous administration the agents may be administered as one composition or as separate compositions, as appropriate. The present invention thus also provides methods for treating a disorder involving cells expressing HER2 as described above, which methods comprise administration of a HER2 antibody of the present invention combined with one or more additional therapeutic agents as described below.

In one embodiment, the present invention provides a method for treating a disorder involving cells expressing HER2 in a subject, which method comprises administration of a therapeutically effective amount of a HER2 antibody of the present invention, and optionally at least one additional therapeutic agent, or an antibody binding to a different epitope than said HER2 antibody, to a subject in need thereof.

In one embodiment, the present invention provides a method for treating or preventing cancer, which method comprises administration of a therapeutically effective amount of a HER2 antibody of the present invention and at least one additional therapeutic agent to a subject in need thereof.

In one embodiment, such an additional therapeutic agent may be selected from an antimetabolite, such as methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, fludarabine, 5-fluorouracil, decarbazine, hydroxyurea, asparaginase, gemcitabine or cladribine.

In another embodiment, such an additional therapeutic agent may be selected from an alkylating agent, such as mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, dacarbazine (DTIC), procarbazine, mitomycin C, cisplatin and other platinum derivatives, such as carboplatin.

In another embodiment, such an additional therapeutic agent may be selected from an anti-mitotic agent, such as taxanes, for instance docetaxel, and paclitaxel, and vinca alkaloids, for instance vindesine, vincristine, vinblastine, and vinorelbine.

In another embodiment, such an additional therapeutic agent may be selected from a topoisomerase inhibitor, such as topotecan or irinotecan, or a cytostatic drug, such as etoposide and teniposide.

In another embodiment, such an additional therapeutic agent may be selected from a growth factor inhibitor, such as an inhibitor of ErbB1 (EGFR) (such as an EGFR antibody, e.g. zalutumumab, cetuximab, panitumumab or nimotuzumab or other EGFR inhibitors, such as gefitinib or erlotinib), another inhibitor of ErbB2 (HER2/neu) (such as a HER2 antibody, e.g. trastuzumab, trastuzumab-DM1 or pertuzumab) or an inhibitor of both EGFR and HER2, such as lapatinib).

In another embodiment, such an additional therapeutic agent may be selected from a tyrosine kinase inhibitor, such as imatinib (Glivec, Gleevec STI571) or lapatinib, PTK787/ZK222584.

In another embodiment, the present invention provides a method for treating a disorder involving cells expressing HER2 in a subject, which method comprises administration of a therapeutically effective amount of an HER2 antibody of the present invention and at least one inhibitor of angiogenesis, neovascularization, and/or other vascularization to a subject in need thereof

Examples of such angiogenesis inhibitors are urokinase inhibitors, matrix metalloprotease inhibitors (such as marimastat, neovastat, BAY 12-9566, AG 3340, BMS-275291 and similar agents), inhibitors of endothelial cell migration and proliferation (such as TNP-470, squalamine, 2-methoxyestradiol, combretastatins, endostatin, angiostatin, penicillamine, SCH66336 (Schering-Plough Corp, Madison, NJ), R115777 (Janssen Pharmaceutica, Inc, Titusville, NJ) and similar agents), antagonists of angiogenic growth factors (such as such as ZD6474, SU6668, antibodies against angiogenic agents

and/or their receptors (such as VEGF (e.g. bevacizumab), bFGF, and angiopoietin-1), thalidomide, thalidomide analogs (such as CC-5013), Sugen 5416, SU5402, antiangiogenic ribozyme (such as angiozyme), interferon  $\alpha$  (such as interferon  $\alpha$ 2a), suramin and similar agents), VEGF-R kinase inhibitors and other anti-angiogenic tyrosine kinase inhibitors (such as SU011248), inhibitors of endothelial-specific integrin/survival signaling (such as vitaxin and similar agents), copper antagonists/chelators (such as tetrathiomolybdate, captopril and similar agents), carboxyamido-triazole (CAI), ABT-627, CM101, interleukin-12 (IL-12), IM862, PNU145156E as well as nucleotide molecules inhibiting angiogenesis (such as antisense-VEGF-cDNA, cDNA coding for angiostatin, cDNA coding for p53 and cDNA coding for deficient VEGF receptor-2).

Other examples of such inhibitors of angiogenesis, neovascularization, and/or other vascularization are anti-angiogenic heparin derivatives (e.g., heparinase III), temozolomide, NK4, macrophage migration inhibitory factor, cyclooxygenase-2 inhibitors, inhibitors of hypoxia-inducible factor 1, anti-angiogenic soy isoflavones, oltipraz, fumagillin and analogs thereof, somatostatin analogues, pentosan polysulfate, tecogalan sodium, dalteparin, tumstatin, thrombospondin, NM-3, combrestatin, canstatin, avastatin, antibodies against other targets, such as anti- $\alpha$ -v/ $\beta$ -3 integrin and anti-kininostatin antibodies.

In one embodiment, a therapeutic agent for use in combination with a HER2 antibody for treating the disorders as described above may be an anti-cancer immunogen, such as a cancer antigen/tumor-associated antigen (e.g., epithelial cell adhesion molecule (EpCAM/TACSTD1), mucin 1 (MUC1), carcinoembryonic antigen (CEA), tumor-associated glycoprotein 72 (TAG-72), gp100, Melan-A, MART-1, KDR, RCAS1, MDA7, cancer-associated viral vaccines (e.g., human papillomavirus vaccines) or tumor-derived heat shock proteins,

In one embodiment, a therapeutic agent for use in combination with a HER2 antibody for treating the disorders as described above may be an anti-cancer cytokine, chemokine, or combination thereof. Examples of suitable cytokines and growth factors include IFN $\gamma$ , IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-18, IL-23, IL-24, IL-27, IL-28a, IL-28b, IL-29, KGF, IFN $\alpha$  (e.g., IFN $\alpha$ 2b), IFN $\beta$ , GM-CSF, CD40L, Flt3 ligand, stem cell factor, ancestim, and TNF $\alpha$ . Suitable chemokines may include Glu-Leu-Arg (ELR)-negative chemokines such as IP-10, MCP-3, MIG, and SDF-1 $\alpha$  from the human CXC and C-C chemokine families. Suitable cytokines include cytokine derivatives, cytokine variants, cytokine fragments, and cytokine fusion proteins.

In one embodiment, a therapeutic agent for use in combination with a HER2 antibody for treating the disorders as described above may be a cell cycle control/apoptosis regulator (or "regulating agent"). A cell cycle control/apoptosis regulator may include molecules that target and modulate cell cycle control/apoptosis regulators such as (i)

cdc-25 (such as NSC 663284), (ii) cyclin-dependent kinases that overstimulate the cell cycle (such as flavopiridol (L868275, HMR1275), 7-hydroxystaurosporine (UCN-01, KW-2401), and roscovitine (R-roscovitine, CYC202)), and (iii) telomerase modulators (such as BIBR1532, SOT-095, GRN163 and compositions described in for instance US 6,440,735 and US 6,713,055). Non-limiting examples of molecules that interfere with apoptotic pathways include TNF-related apoptosis-inducing ligand (TRAIL)/apoptosis-2 ligand (Apo-2L), antibodies that activate TRAIL receptors, IFNs, and anti-sense Bcl-2.

In one embodiment, a therapeutic agent for use in combination with a HER2 antibody for treating the disorders as described above may be a hormonal regulating agent, such as agents useful for anti-androgen and anti-estrogen therapy. Examples of such hormonal regulating agents are tamoxifen, idoxifene, fulvestrant, droloxifene, toremifene, raloxifene, diethylstilbestrol, ethinyl estradiol/estinyl, an antiandrogene (such as flutaminde/eulexin), a progestin (such as such as hydroxyprogesterone caproate, medroxy-progesterone/provera, megestrol acepate/megace), an adrenocorticosteroid (such as hydrocortisone, prednisone), luteinizing hormone-releasing hormone (and analogs thereof and other LHRH agonists such as buserelin and goserelin), an aromatase inhibitor (such as anastrozole/arimidex, aminoglutethimide/cytraden, exemestane) or a hormone inhibitor (such as octreotide/sandostatin).

In one embodiment, a therapeutic agent for use in combination with a HER2 antibody for treating the disorders as described above may be an anti-anergic agent, such as compounds are molecules that block the activity of CTLA-4, e.g. ipilimumab.

In one embodiment, a therapeutic agent for use in combination with a HER2 antibody for treating the disorders as described above may be an anti-cancer nucleic acid or an anti-cancer inhibitory RNA molecule.

Examples of other anti-cancer agents, which may be relevant as therapeutic agents for use in combination with a HER2 antibody for treating the disorders as described above are differentiation inducing agents, retinoic acid analogues (such as all trans retinoic acid, 13-cis retinoic acid and similar agents), vitamin D analogues (such as seocalcitol and similar agents), inhibitors of ErbB3, ErbB4, IGF-IR, insulin receptor, PDGFRa, PDGFRbeta, Flk2, Flt4, FGFR1, FGFR2, FGFR3, FGFR4, TRKA, TRKC, RON (such as an anti-RON antibody), Sea, Tie, Tie2, Eph, Ret, Ros, Alk, LTK, PTK7 and similar agents.

Examples of other anti-cancer agents, which may be relevant as therapeutic agents for use in combination with a HER2 antibody for treating the disorders as described above are estramustine and epirubicin.

Examples of other anti-cancer agents, which may be relevant as therapeutic agents for use in combination with a HER2 antibody for treating the disorders as described above

are a HSP90 inhibitor like 17-allyl amino geldanamycin, antibodies directed against a tumor antigen such as PSA, CA125, KSA, integrins, e.g. Integrin  $\beta$ 1, or inhibitors of VCAM.

Examples of other anti-cancer agents, which may be relevant as therapeutic agents for use in combination with a HER2 antibody for treating the disorders as described above are calcineurin-inhibitors (such as valspodar, PSC 833 and other MDR-1 or p-glycoprotein inhibitors), TOR-inhibitors (such as sirolimus, everolimus and rapamycin), and inhibitors of "lymphocyte homing" mechanisms (such as FTY720), and agents with effects on cell signaling such as adhesion molecule inhibitors (for instance anti-LFA).

In one embodiment, the HER2 antibody of the invention is for use in combination with one or more other therapeutic antibodies, such as ofatumumab, zanolimumab, daratumumab, ranibizumab, nimotuzumab, panitumumab, hu806, daclizumab (Zenapax™), basiliximab (Simulect™), infliximab (Remicade™), adalimumab (Humira™), natalizumab (Tysabri™), omalizumab (Xolair™), efalizumab (Raptiva™) and/or rituximab.

In another embodiment, two or more different antibodies of the invention as described herein are used in combination for the treatment of disease. Particularly interesting combinations include two or more non-blocking antibodies. Such combination therapy may lead to binding of an increased number of antibody molecules per cell, which may give increase efficacy, e.g. via activation of complement-mediated lysis.

In addition to the above, other embodiments of combination therapies of the invention include the following:

For the treatment of breast cancer, a HER2 antibody or a therapeutic conjugate thereof, in combination with methotrexate, paclitaxel, doxorubicin, carboplatin, cyclophosphamide, daunorubicin, epirubicin, 5-fluorouracil, gemcitabine, ixabepilone, mutamycin, mitoxantrone, vinorelbine, docetaxel, thiotepa, vincristine, capecitabine, an EGFR antibody (e.g. zalutumumab, cetuximab, panitumumab or nimotuzumab) or other EGFR inhibitor (such as gefitinib or erlotinib), another HER2 antibody or -conjugate (such as, e.g., trastuzumab, trastuzumab-DM1 or pertuzumab), an inhibitor of both EGFR and HER2 (such as lapatinib), and/or in combination with a HER3 inhibitor.

For the treatment of non-small-cell lung cancer, a HER2 antibody in combination with EGFR inhibitors, such as an EGFR antibody, e.g. zalutumumab, cetuximab, panitumumab or nimotuzumab or other EGFR inhibitors (such as gefitinib or erlotinib), or in combination with an another HER2 agent (such as a HER2 antibody, e.g. trastuzumab, trastuzumab-DM1 or pertuzumab) or in combination with an inhibitor of both EGFR and HER2, such as lapatinib, or in combination with a HER3 inhibitor.

For the treatment of colorectal cancer a HER2 antibody in combination with one or more compounds selected from: gemcitabine, bevacizumab, FOLFOX, FOLFIRI, XELOX, IFL,

oxaliplatin, irinotecan, 5-FU/LV, Capecitabine, UFT, EGFR targeting agents, such as cetuximab, panitumumab, zalutumumab; VEGF inhibitors, or tyrosine kinase inhibitors such as sunitinib.

For the treatment of prostate cancer a HER2 antibody in combination with one or more compounds selected from: hormonal/antihormonal therapies; such as antiandrogens, Luteinizing hormone releasing hormone (LHRH) agonists, and chemotherapeutics such as taxanes, mitoxantrone, estramustine, 5FU, vinblastine, and ixabepilone.

#### Radiotherapy - surgery

In one embodiment, the present invention provides a method for treating a disorder involving cells expressing HER2 in a subject, which method comprises administration of a therapeutically effective amount of a HER2 antibody, such as a HER2 antibody of the present invention, and radiotherapy to a subject in need thereof.

In one embodiment, the present invention provides a method for treating or preventing cancer, which method comprises administration of a therapeutically effective amount of a HER2 antibody, such as a HER2 antibody of the present invention, and radiotherapy to a subject in need thereof.

In one embodiment, the present invention provides the use of a HER2 antibody, such as a HER2 antibody of the present invention, for the preparation of a pharmaceutical composition for treating cancer to be administered in combination with radiotherapy.

Radiotherapy may comprise radiation or associated administration of radiopharmaceuticals to a patient is provided. The source of radiation may be either external or internal to the patient being treated (radiation treatment may, for example, be in the form of external beam radiation therapy (EBRT) or brachytherapy (BT)). Radioactive elements that may be used in practicing such methods include, e.g., radium, cesium-137, iridium-192, americium-241, gold-198, cobalt-57, copper-67, technetium-99, iodide-123, iodide-131, and indium-111.

In a further embodiment, the present invention provides a method for treating or preventing cancer, which method comprises administration to a subject in need thereof of a therapeutically effective amount of a HER2 antibody, such as a HER2 antibody of the present invention, in combination with surgery.

#### Diagnostic uses

The HER2 antibodies of the invention may also be used for diagnostic purposes. Thus, in a further aspect, the invention relates to a diagnostic composition comprising a HER2 antibody as defined herein.

In one embodiment, the HER2 antibodies of the present invention may be used *in vivo* or *in vitro* for diagnosing diseases wherein activated cells expressing HER2 play an active role in the pathogenesis, by detecting levels of HER2, or levels of cells which contain HER2 on their membrane surface. This may be achieved, for example, by contacting a sample to be tested, optionally along with a control sample, with the HER2 antibody under conditions that allow for formation of a complex between the antibody and HER2.

Thus, in a further aspect, the invention relates to a method for detecting the presence of HER2 antigen, or a cell expressing HER2, in a sample comprising:

- contacting the sample with a HER2 antibody of the invention under conditions that allow for formation of a complex between the antibody and HER2; and
- analyzing whether a complex has been formed.

In one embodiment, the method is performed *in vitro*.

More specifically, the present invention provides methods for the identification of, and diagnosis of invasive cells and tissues, and other cells targeted by HER2 antibodies of the present invention, and for the monitoring of the progress of therapeutic treatments, status after treatment, risk of developing cancer, cancer progression, and the like.

Suitable labels for the HER2 antibody and/or secondary antibodies used in such techniques are well-known in the art.

In a further aspect, the invention relates to a kit for detecting the presence of HER2 antigen, or a cell expressing HER2, in a sample comprising

- a HER2 antibody of the invention or a bispecific molecule of the invention; and
- instructions for use of the kit.

In one embodiment, the present invention provides a kit for diagnosis of cancer comprising a container comprising a HER2 antibody, and one or more reagents for detecting binding of the HER2 antibody to HER2. Reagents may include, for example, fluorescent tags, enzymatic tags, or other detectable tags. The reagents may also include secondary or tertiary antibodies or reagents for enzymatic reactions, wherein the enzymatic reactions produce a product that may be visualized.

#### Anti-idiotypic antibodies

In a further aspect, the invention relates to an anti-idiotypic antibody which binds to a HER2 antibody of the invention as described herein.

An anti-idiotypic (Id) antibody is an antibody which recognizes unique determinants generally associated with the antigen-binding site of an antibody. An Id antibody may be prepared by immunizing an animal of the same species and genetic type as the source of a HER2 mAb with the mAb to which an anti-Id is being prepared. The immunized animal

typically can recognize and respond to the idiotypic determinants of the immunizing antibody by producing an antibody to these idiotypic determinants (the anti-Id antibody).

An anti-Id antibody may also be used as an "immunogen" to induce an immune response in yet another animal, producing a so-called anti-anti-Id antibody. An anti-anti-Id may be epitopically identical to the original mAb, which induced the anti-Id. Thus, by using antibodies to the idiotypic determinants of a mAb, it is possible to identify other clones expressing antibodies of identical specificity.

The present invention is further illustrated by the following examples, which should not be construed as limiting the scope of the invention.

The present invention is further illustrated by the following examples, which should not be construed as limiting the scope of the invention.

## **EXAMPLES**

### **Example 1 - Expression constructs for HER2 and HER2 variants**

Fully codon-optimized constructs for expression of full length HER2 (1255 aa, Swissprot P04626), the extracellular domain (ECD) of HER2 (Her2-ECDHis, aa 1-653 with a C-terminal His6 tag), the naturally occurring HER2 splice variant (Her2-delex16, resulting from exon 16 deletion and lacking aa 633-648) and a truncated form of the HER2 receptor (Her2-stumpy, aa 648-1256), were generated. The construct contained suitable restriction sites for cloning and an optimal Kozak sequence (Kozak, M., *Gene* 1999;234(2):187-208.). The constructs were cloned in the mammalian expression vector pEE13.4 (Lonza Biologics; Bebbington, C.R., *et al.*, *Biotechnology (N Y)* 1992;10(2):169-75) and fully sequenced to confirm the correctness of the construct.

### **Example 2 - Expression constructs for Pertuzumab, C1 and F5**

Fully codon-optimized constructs for expression of the heavy chain (HC) and the light chain (LC) of the IgG1 antibodies pertuzumab, C1 and F5 in HEK cells, were generated. The variable regions encoded by these constructs are identical to those described in U.S. Patent No. 6,949,245 for pertuzumab heavy chain and light chain and U.S. Patent No. 7,244,826 for C1 and F5 heavy and light chain. For C1 and F5, the mammalian expression vectors p33G1f and p33K or p33L (pcDNA3.3 (Invitrogen)) containing the fully codon optimized constant region for the human IgG1 heavy chain (allotype f), the human kappa light chain or the human lambda light chain, respectively, were used. For pertuzumab, the mammalian expression vectors pG1f (pEE12.4 (Lonza Biologics) and pKappa (pEE6.4 (Lonza Biologics), containing the fully codon-optimized constant region for the human IgG1 heavy chain (allotype f) and the human kappa light chain, respectively, were used.

Trastuzumab (Herceptin®) can be produced in the same manner, using the heavy and light chain sequences described in, e.g., U.S. Patent No. 7,632,924.

### **Example 3 - Transient expression in HEK-293 or CHO cells**

Freestyle™ 293-F (a HEK-293 subclone adapted to suspension growth and chemically defined Freestyle medium, (HEK-293F)) cells were obtained from Invitrogen and transfected with the appropriate plasmid DNA, using 293fectin™ (Invitrogen) according to the manufacturer's instructions. In the case of antibody expression, the appropriate heavy chain and light chain expression vectors were co-expressed.

pEE13.4Her2, pEE13.4Her2-delex16 and pEE13.4Her2-stumpy were transiently transfected in the Freestyle™ CHO-S (Invitrogen) cell line using Freestyle MAX™ transfection reagent (Invitrogen). Expression of HER2 and Her2-delex16 was tested by means of FACS analysis as described below.

### **Example 4 - Stable polyclonal pool expression in NS0**

pEE13.4Her2, pEE13.4Her2-delex16 and pEE13.4Her2-stumpy were stably transfected in NS0 cells by nucleofection (Amaxa). A pool of stably transfected cells was established after selection on glutamine dependent growth, based on the integrated glutamine synthetase selection marker (Barnes, L.M., *et al.*, Cytotechnology 2000;32(2):109-123).

### **Example 5 - Purification of His-tagged HER2**

Her2ECDHis was expressed in HEK-293F cells. The His-tag in Her2ECDHis enabled purification with immobilized metal affinity chromatography, since the His-tagged protein binds strongly to the resin beads, while other proteins present in the culture supernatant do not bind strongly.

In this process, a chelator fixed onto the chromatographic resin was charged with Co<sup>2+</sup> cations. Her2ECDHis containing supernatant was incubated with the resin in batch mode (i.e. solution). After incubation, the beads were retrieved from the supernatant and packed into a column. The column was washed in order to remove weakly bound proteins. The strongly bound Her2ECDHis proteins were then eluted with a buffer containing imidazole, which competes with the binding of His to Co<sup>2+</sup>. The eluent was removed from the protein by buffer exchange on a desalting column.

### **Example 6 - Immunization procedure of transgenic mice**

Antibodies 001, 019, 021, 025, 027, 032, 033, 035, 036, 049, 050, 051, 054, 055, 084, 091, 094, 098, 100, 105, 123 and 124 were derived from the following immunization: three

female HCo12 mice, one male and two female HCo12-Balb/C mice, one male HCo17 mouse and one male HCo20 mouse (Medarex, San José, CA, USA) were immunized alternating with  $5 \times 10^6$  NS0 cells transiently transfected with Her2ECD intraperitoneal (IP) and 20  $\mu$ g Her2ECDHis protein coupled to the hapten Keyhole Limpet Hemocyanin (KLH) subcutaneous (SC) at the tail base, with an interval of fourteen days. A maximum of eight immunizations was performed per mouse (four IP and four SC immunizations). The first immunization with cells was done in complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI, USA). For all other immunizations, cells were injected IP in PBS and KLH coupled Her2ECD was injected SC using incomplete Freund's adjuvant (IFA; Difco Laboratories, Detroit, MI, USA).

Antibodies 125, 127, 129, 132, 152, 153 and 159 were derived from the following immunization: one male and two female HCo12-Balb/C mice, one female HCo20 mouse, and one female HCo12 mouse (Medarex) were immunized alternating with  $5 \times 10^6$  NS0 cells transiently transfected with Her2delex16 IP and 20  $\mu$ g Her2ECDHis protein coupled to the hapten Keyhole Limpet Hemocyanin (KLH) SC at the tail base, with an interval of fourteen days. A maximum of eight immunizations was performed per mouse (four IP and four SC immunizations). The first immunization with cells was done in complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI, USA). For all other immunizations, cells were injected IP in PBS and KLH coupled Her2ECD was injected SC using incomplete Freund's adjuvant (IFA; Difco Laboratories, Detroit, MI, USA).

Antibody 143, 160, 161, 162, 166 and 169 were derived from the following immunization: one female and one male HCo12 mouse, one female HCo12-Balb/C mouse, one male HCo17 mouse and one male HCo20 mouse (Medarex) were immunized alternating with 20  $\mu$ g Her2ECDHis protein coupled to the hapten Keyhole Limpet Hemocyanin (KLH), alternating IP and SC at the tail base with an interval of fourteen days. A maximum of eight immunizations was performed per mouse (four IP and four SC immunizations). The first immunization was done IP in complete Freund's adjuvant (CFA; Difco Laboratories, Detroit, MI, USA). The other immunizations were injected using incomplete Freund's adjuvant (IFA; Difco Laboratories, Detroit, MI, USA).

Mice with at least two sequential titers against TC1014-Her2, TC1014-Her2delex16 or TC1014-Her2stumpy in the antigen specific FMAT screening assay (as described in example 7), were considered positive and fused.

#### **Example 7 - Homogeneous antigen specific screening assay**

The presence of HER2 antibodies in sera of immunized mice or HuMab (human monoclonal antibody) hybridoma or transfectoma culture supernatant was determined by homogeneous antigen specific screening assays (four quadrant) using Fluorometric Micro volume Assay

Technology (FMAT; Applied Biosystems, Foster City, CA, USA). For this, a combination of 4 cell based assays was used. Binding to TC1014-Her2 (HEK-293F cells transiently expressing the HER2 receptor; produced as described above), TC1014-Her2delex16 (HEK-293F cells transiently expressing the extracellular domain of Her2-delex (a 16 amino acid deletion mutant of the HER2 receptor; produced as described above) and TC1014-Her2stumpy (HEK-293F cells transiently expressing the extracellular stumpy domain of the HER2 receptor; produced as described above) as well as HEK293 wild type cells (negative control cells which do not express HER2) was determined. Samples were added to the cells to allow binding to HER2. Subsequently, binding of HuMab was detected using a fluorescent conjugate (Goat anti-Human IgG-Cy5; Jackson ImmunoResearch). TH1014-Pertuzumab (produced in HEK-293F cells) was used as a positive control and HuMab-mouse pooled serum and HuMab-KLH were used as negative controls. The samples were scanned using an Applied Biosystems 8200 Cellular Detection System (8200 CDS) and 'counts x fluorescence' was used as read-out. Samples were stated positive when counts were higher than 50 and counts x fluorescence were at least three times higher than the negative control.

#### **Example 8 - HuMab hybridoma generation**

HuMab mice with sufficient antigen-specific titer development (defined as above) were sacrificed and the spleen and lymph nodes flanking the abdominal aorta and vena cava were collected. Fusion of splenocytes and lymph node cells to a mouse myeloma cell line was done by electrofusion using a CEEF 50 Electrofusion System™ (Cyto Pulse Sciences, Glen Burnie, MD, USA), essentially according to the manufacturer's instructions. Next, the primary wells were sub cloned using the ClonePix™ system (Genetix, Hampshire, UK). To this end specific primary well hybridoma's were seeded in semisolid medium made from 40% CloneMedia™ (Genetix, Hampshire, UK) and 60% HyQ 2x™ complete media (Hyclone, Waltham, USA). The sub clones were retested in the antigen-specific binding assay as described in Example 7 and IgG levels were measured using an Octet™ (ForteBio, Menlo Park, USA) in order to select the most specific and best producing clone per primary well for further expansion. Further expansion and culturing of the resulting HuMab hybridomas were done based upon standard protocols (e.g. as described in Coligan J.E., Bierer, B.E., Margulies, D.H., Shevach, E.M. and Strober, W., eds. Current Protocols in Immunology, John Wiley & Sons, Inc., 2006). Clones derived by this process were designated PC1014.

#### **Example 9 - Mass Spectrometry of purified antibodies**

Small aliquots of 0.8 mL antibody containing supernatant from 6-well or Hyperflask™ stage were purified using PhyTip™ columns containing Protein G resin (PhyNexus Inc., San Jose,

USA) on a Sciclone™ ALH 3000 workstation (Caliper Lifesciences, Hopkinton, USA). The PhyTip columns were used according to manufacturer's instructions, although buffers were replaced by: Binding Buffer PBS (B.Braun, Medical B.V., Oss, Netherlands) and Elution Buffer 0.1M Glycine-HCl pH 2.7 (Fluka Riedel-de Haën, Buchs, Germany). After purification, samples were neutralized with 2M Tris-HCl, pH 9.0 (Sigma-Aldrich, Zwijndrecht, Netherlands). Alternatively, in some cases larger volumes of culture supernatant were purified using MabSelect SuRe™.

After purification, the samples were placed in a 384-well plate (Waters, 100 µl square well plate, part# 186002631). Samples were deglycosylated overnight at 37°C with N-glycosidase F (Roche cat no 11365177001. DTT (15 mg/mL) was added (1 µL/well) and incubated for 1 h at 37°C. Samples (5 or 6 µL) were desalted on an Acquity UPLC™ (Waters, Milford, USA) with a BEH300 C18, 1.7µm, 2.1x 50 mm column at 60 °C. MQ water and LC-MS grade acetonitrile (Biosolve, cat no 01204101, Valkenswaard, The Netherlands) with both 0.1% formic acid (Fluka, cat no 56302, Buchs, Germany), were used as Eluents A and B, respectively. Time-of-flight electrospray ionization mass spectra were recorded on-line on a micrOTOF™ mass spectrometer (Bruker, Bremen, Germany) operating in the positive ion mode. Prior to analysis, a 900-3000 m/z scale was calibrated with ES tuning mix (Agilent Technologies, Santa Clara, USA). Mass spectra were deconvoluted with DataAnalysis™ software v. 3.4 (Bruker) using the Maximal Entropy algorithm searching for molecular weights between 5 and 80 kDa.

After deconvolution, the resulting heavy and light chain masses for all samples were compared in order to find duplicate antibodies. This was sometimes due to the presence of an extra light chain, but in the comparison of the heavy chains, the possible presence of C-terminal lysine variants was also taken into account. This resulted in a list of unique antibodies, i.e., a unique combination of specific heavy and light chains. In case duplicate antibodies were found, one unique antibody was selected based on results from other tests.

#### **Example 10 - Sequence analysis of the HER2 antibody variable domains and cloning in expression vectors**

Total RNA of the HER2 HuMabs was prepared from 5x10<sup>6</sup> hybridoma cells and 5'-RACE-Complementary DNA (cDNA) was prepared from 100 ng total RNA, using the SMART RACE™ cDNA Amplification kit (Clontech), according to the manufacturer's instructions. VH and VL coding regions were amplified by PCR and cloned directly, in frame, in the pG1f and pKappa expression vectors, by ligation independent cloning (Aslanidis, C. and P.J. de Jong, Nucleic Acids Res 1990;18(20): 6069-74). Clones derived by this process were designated TH1014. For each antibody, 16 VL clones and 8 VH clones were sequenced. Clones which predicted

heavy and light chain mass in agreement with the mass of the hybridoma derived material of the same antibody (as determined by mass spectrometry) were selected for further study and expression.

The resulting sequences are shown in Figures 1 and 2 and in the Sequence Listing. Selected sequences are also described in more detail below. CDR sequences were defined according to IMGT (Lefranc MP. *et al.*, Nucleic Acids Research, 27, 209-212, 1999 and Brochet X. Nucl. Acids Res. 36, W503-508 (2008)). Table 1, Table 2 and Table 3 give an overview of antibody sequence information or germline sequences, and Table 4 shows consensus sequences.

**Table 1:** Heavy chain variable region (VH), light chain variable region (VL) and CDR sequences of HuMabs 169, 050, 084, 025, 091, 129, 127, 159, 098, 153, and 132.

|              |              |                                                                                                                                       |
|--------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No:1  | VH 169       | QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISW<br>VRQAPGQGLEWMGWLSAYSGNTIYAQKLQGRVTMT<br>TDTSTTTAYMELRSLRSDDTAVYYCARDRIVVRPDYF<br>DYWGQGTLLTVSS |
| SEQ ID No:2  | VH 169, CDR1 | GYTFTNYG                                                                                                                              |
| SEQ ID No:3  | VH 169, CDR2 | LSAYSGNT                                                                                                                              |
| SEQ ID No:4  | VH 169, CDR3 | ARDRIVVRPDYFDY                                                                                                                        |
| SEQ ID No:5  | VL 169       | EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQ<br>QKPGQAPRLLIYDASNRAITGIPARFSGSGSGTDFTLI<br>SSLEPEDFAVYYCQQRSNWPRTFGQGTRLEIK                   |
| SEQ ID No:6  | VL 169, CDR1 | QSVSSY                                                                                                                                |
|              | VL 169, CDR2 | DAS                                                                                                                                   |
| SEQ ID No:7  | VL 169, CDR3 | QQRSNWPRT                                                                                                                             |
| SEQ ID No:8  | VH 050       | EVQLLESQGGGLVQPGGSLRLSCAASGFTFSSYAMNW<br>VRQAPGKGLEWVSAISGRGGTTYADSVKGRFTISR<br>DNSKNTLYLQMSSLRAEDTAVYYCAKARANWDYFDY<br>WGQGTLLTVSS   |
| SEQ ID No:9  | VH 050, CDR1 | GFTFSSYA                                                                                                                              |
| SEQ ID No:10 | VH 050, CDR2 | ISGRGGTT                                                                                                                              |
| SEQ ID No:11 | VH 050, CDR3 | AKARANWDYFDY                                                                                                                          |
| SEQ ID No:12 | VL 050       | DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWY<br>QHKPGKAPKLLIYAASILQSGVPSRFSGSGSGTDFTL<br>TISSLQPEDFATYYCQQANSFPITFGQGRLEIK                    |
| SEQ ID No:13 | VL 050, CDR1 | QGISSW                                                                                                                                |
|              | VL 050, CDR2 | AAS                                                                                                                                   |
| SEQ ID No:14 | VL 050, CDR3 | QQANSFPIT                                                                                                                             |

|               |              |                                                                                                                                       |
|---------------|--------------|---------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No:15  | VH 084       | QVQLVQSGAEVKKPGSSVKVSCKASGGTFRTYAINW<br>VRQAPGQGLEWMGRINTVLGIVNHAQKFQGRVTITA<br>DKSTNTAYMELNSLRSEDTAVYYCAREKGVDYYYGIE<br>VWGQGTTVTVSS |
| SEQ ID No:16  | VH 084, CDR1 | GGTFRTYA                                                                                                                              |
| SEQ ID No:17  | VH 084, CDR2 | INTVLGIV                                                                                                                              |
| SEQ ID No:18  | VH 084, CDR3 | AREKGVDYYYGIEV                                                                                                                        |
| SEQ ID No:19  | VL 084       | DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWY<br>QHKPGKAPKLLIYVASTLQSGVPSRFSGSGSGTDFTL<br>TISSLQPEDFATYYCQQANSFPLTFGGGTKEIK                    |
| SEQ ID No:20  | VL 084, CDR1 | QGISSW                                                                                                                                |
|               | VL 084, CDR2 | VAS                                                                                                                                   |
| SEQ ID No:21  | VL 084, CDR3 | QQANSFPLT                                                                                                                             |
| SEQ ID No:22  | VH 025       | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWN<br>WIRQPPGKGLEWIGEIHSGSTNYPNPSLKSRVTISVD<br>TSKNQFSLKLSSVTAADTAVYYCARGYYDSGVYYFDY<br>WAQGTLLTVSS  |
| SEQ ID No:23  | VH 025, CDR1 | GGSFSDYY                                                                                                                              |
| SEQ ID No:24  | VH 025, CDR2 | IHHSGST                                                                                                                               |
| SEQ ID No:25  | VH 025, CDR3 | ARGYYDSGVYYFDY                                                                                                                        |
| SEQ ID No:26  | VL 025       | DIQMTQSPSSLSASVGDRVTITCRASQGISRWLAWY<br>QQKPEKAPKSLIYAASSLRSGVPSRFSGSGSGTDFTL<br>TISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK                   |
| SEQ ID No:27  | VL 025, CDR1 | QGISRW                                                                                                                                |
|               | VL 025, CDR2 | AAS                                                                                                                                   |
| SEQ ID No:28  | VL 025, CDR3 | QQYNSYPIT                                                                                                                             |
| SEQ ID No:29  | VH 091       | QVQLQQWGAGLLKPSETLSLTCAVSGGSFSGYYWT<br>WIRQPPGKGLEWIGEIYHSGDTNYPNPSLKSRVTISVD<br>TSKNQFSLKLYSVTAADTAVYYCARLYFGSGIYYLDY<br>WGQGTLLTVSS |
| SEQ ID No:30  | VH 091, CDR1 | GGSFSGYY                                                                                                                              |
| SEQ ID No:163 | VH 091, CDR2 | IYHSGDT                                                                                                                               |
| SEQ ID No:31  | VH 091, CDR3 | ARLYFGSGIYYLDY                                                                                                                        |
| SEQ ID No:32  | VL 091       | DIQMTQSPSSLSASVGDRVTITCRASQGISSWLWVY<br>QQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTL<br>TISSLQPEDFATYYCQQYNSFPPTFGQGTKEIK                    |
| SEQ ID No:33  | VL 091, CDR1 | QGISSW                                                                                                                                |
|               | VL 091, CDR2 | AAS                                                                                                                                   |
| SEQ ID No:34  | VL 091, CDR3 | QQYNSFPPT                                                                                                                             |
| SEQ ID No:35  | VH 129       | QVQLVESGGGVVQPGRSLRLSCAASGFTFSTFAIHW<br>VRQAPGKGLEWVAVISYDGGHKFYADSVKGRFTISR<br>DNSKNTLYLQMNSLRAEDTAMYYCARGLGWGAFD<br>YWQGTLLTVSS     |
| SEQ ID No:36  | VH 129, CDR1 | GFTFSTFA                                                                                                                              |

|              |              |                                                                                                                                          |
|--------------|--------------|------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No:37 | VH 129, CDR2 | ISYDGGHK                                                                                                                                 |
| SEQ ID No:38 | VH 129, CDR3 | ARGLGVWGAFDY                                                                                                                             |
| SEQ ID No:39 | VL 129       | EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQ<br>QKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLT<br>SSLEPEDFAVYYCQQRSNWWTFGQGTKVEIK                        |
| SEQ ID No:40 | VL 129, CDR1 | QSVSSY                                                                                                                                   |
|              | VL 129, CDR2 | DAS                                                                                                                                      |
| SEQ ID No:41 | VL 129, CDR3 | QQRSNWWT                                                                                                                                 |
| SEQ ID No:42 | VH 127       | EVQLVQSGAEVKKPGESLTISCKGSGYSFSIYWIGW<br>VRQMPGKGLEWMGIIFPGDSDIRYSPSFQGQVTISA<br>DKSISTAYLQWSSLKASDTAMYYCARQPGDWSPRH<br>WYFDLWGRGTLTVSS   |
| SEQ ID No:43 | VH 127, CDR1 | GYSFSIYW                                                                                                                                 |
| SEQ ID No:44 | VH 127, CDR2 | IFPGSDSI                                                                                                                                 |
| SEQ ID No:45 | VH 127, CDR3 | ARQPGDWSPRHWYFDL                                                                                                                         |
| SEQ ID No:46 | VL 127       | VIWMTQSPSLLSASTGDRVTISCRMSQGISSYLAWY<br>QQKPGKAPELLIYAASLTQSGVPSRFSGSGSGTDFTL<br>TISYLQSEDFATYYCQYYSFPLTFGGGTKVEIK                       |
| SEQ ID No:47 | VL 127, CDR1 | QGISSY                                                                                                                                   |
|              | VL 127, CDR2 | AAS                                                                                                                                      |
| SEQ ID No:48 | VL 127, CDR3 | QYYSFPLT                                                                                                                                 |
| SEQ ID No:49 | VH 159       | EVQLVQSGAEVKKPGESLKISCKGSGYNFTSYWIGW<br>VRQMPGKGLEWMGIIPGDSDTRYSPSFQGQVTISA<br>DKSISTAYLQWSSLKASDTAMYYCARWGTYDILT<br>GFNWFDPWGQGTTLTVSS  |
| SEQ ID No:50 | VH 159, CDR1 | GYNFTSYW                                                                                                                                 |
| SEQ ID No:51 | VH 159, CDR2 | IYPGDSDT                                                                                                                                 |
| SEQ ID No:52 | VH 159, CDR3 | ARWGTYDILTYFN                                                                                                                            |
| SEQ ID No:53 | VL 159       | DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWY<br>QQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTL<br>TISSLQPEDFATYYCQYYIYPWTFGQGTKVEIK                       |
| SEQ ID No:54 | VL 159, CDR1 | QGISSW                                                                                                                                   |
|              | VL 159, CDR2 | AAS                                                                                                                                      |
| SEQ ID No:55 | VL 159, CDR3 | QYYIYPWT                                                                                                                                 |
| SEQ ID No:56 | VH 098       | EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMSW<br>VRQAPGKGLEWVSAISGSAYSTYYADSVKGRFTISR<br>DNSKNTLWLQMNSLRAEDTAVYYCAKAHYHGSGSY<br>TLFDYWGGQGTTLTVSS |
| SEQ ID No:57 | VH 098, CDR1 | GFTFSNYG                                                                                                                                 |
| SEQ ID No:58 | VH 098, CDR2 | ISGSAYST                                                                                                                                 |
| SEQ ID No:59 | VH 098, CDR3 | AKAHYHGSGSYTLFDY                                                                                                                         |

|              |              |                                                                                                                                          |
|--------------|--------------|------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No:60 | VL 098       | DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWY<br>QQKPEKAPKSLIYAASSLQSGVPSRFSGSGSGTDFTL<br>TISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK                      |
| SEQ ID No:61 | VL 098, CDR1 | QGISSW                                                                                                                                   |
|              | VL 098, CDR2 | AAS                                                                                                                                      |
| SEQ ID No:62 | VL 098, CDR3 | QQYNSYPYT                                                                                                                                |
| SEQ ID No:63 | VH 153       | QVQLVESGGGVVQPGRSLRLSCAASGFTFSDYVIHW<br>VRQAPGKGLEWVTVISYDGSNKYYADSVKGRFTISR<br>DNSKNTLYLQMNSLSAEDTAMYYCARGGITGTTGVF<br>DYWGQGTLLTVSS    |
| SEQ ID No:64 | VH 153, CDR1 | GFTFSDYV                                                                                                                                 |
| SEQ ID No:65 | VH 153, CDR2 | ISYDGSNK                                                                                                                                 |
| SEQ ID No:66 | VH 153, CDR3 | ARGGITGTTGVFDY                                                                                                                           |
| SEQ ID No:67 | VL 153       | DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWY<br>QQKPEKAPKSLIYDASSLQSGVPSRFSGSGYGTDFSL<br>TISSLQPEDFAIYYCQQYKSYPTFGQGTRLEIK                       |
| SEQ ID No:68 | VL 153, CDR1 | QGISSW                                                                                                                                   |
|              | VL 153, CDR2 | DAS                                                                                                                                      |
| SEQ ID No:69 | VL 153, CDR3 | QQYKSYPT                                                                                                                                 |
| SEQ ID No:70 | VH 132       | QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISW<br>VRQAPGQGLEWMGWISAYNGNSNYVQKFQGRVTM<br>TTDTTSTAYMELRSLTSDDTAVYYCAREYSYDSGT<br>FYGMDEVWGQGTITVTVSS |
| SEQ ID No:71 | VH 132, CDR1 | GYTFTSYG                                                                                                                                 |
| SEQ ID No:72 | VH 132, CDR2 | ISAYNGNS                                                                                                                                 |
| SEQ ID No:73 | VH 132, CDR3 | AREYSYDSGTIFYFYGMDEV                                                                                                                     |
| SEQ ID No:74 | VL 132       | EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQ<br>QKPGQAPRLIYDASNRAITGIPARFSGSGSGTDFTLTI<br>SSLEPEDFAVYYCQQRSNWPMYTFGQGTKLEIK                     |
| SEQ ID No:75 | VL 132, CDR1 | QSVSSY                                                                                                                                   |
|              | VL 132, CDR2 | DAS                                                                                                                                      |
| SEQ ID No:76 | VL 132, CDR3 | QQRSNWPMYT                                                                                                                               |

**Table 2:** Mouse origin and heavy and light chain sequence homologies of selected HuMabs.

| HuMab: | Mouse: | Strain:     | Germline VH: | Germline VL: |
|--------|--------|-------------|--------------|--------------|
| 169    | 361494 | HCo20       | IgHV1-18-01  | IgKV3-11-01  |
| 050    | 350633 | HCo12       | IgHV3-23-01  | IgKV1-12-01  |
| 084    | 350615 | HCo12-Balbc | IgHV1-69-04  | IgKV1-12-01  |
| 025    | 350631 | HCo12       | IgHV4-34-01  | IgKV1D-16-01 |
| 091    | 350630 | HCo12       | IgHV4-34-01  | IgKV1D-16-01 |

|     |        |             |               |              |
|-----|--------|-------------|---------------|--------------|
| 129 | 359783 | HCo12-Balbc | IgHV3-30-3-01 | IgKV3-11-01  |
| 127 | 359783 | HCo12-Balbc | IgHV5-51-01   | IgKV1D-8-01  |
| 159 | 363503 | HCo12       | IgHV5-51-01   | IgKV1D-16-01 |
| 098 | 350659 | HCo17       | IgHV3-23-01   | IgKV1D-16-01 |
| 153 | 359785 | HCo12-Balbc | IgHV3-30-3-01 | IgKV1D-16-01 |
| 132 | 361487 | HCo20       | IgHV1-18-01   | IgKV3-11-01  |

**Table 3:** Heavy chain variable region (VH), light chain variable region (VL) sequences of HuMabs 049, 051, 055, 123, 161, 124, 001, 143, 019, 021, 027, 032, 035, 036, 054, 094. The respective CDRs correspond to those underlined in Figures 1 and 2, for VH and VL sequences, respectively.

|               |        |                                                                                                                                    |
|---------------|--------|------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No: 77 | VH 049 | EVQLLESGGDLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG<br>KGLEWVSAISGRGGTTYADSVKGRFTISRDNSKSTLCLQMNS<br>LRAEDTAVYYCAKARANWDYFDYWGQGT LTVSS     |
| SEQ ID No: 78 | VL 049 | DIQMTQSPSSVSASVGDRVITICRASQGISSWLAWYQHKPGK<br>APKLLIYAASILQSGVPSRFSGSGSGTDFTLTISSLRPEDFATYY<br>CQQANSFPITFGQGT RLEIK               |
| SEQ ID No: 79 | VH 051 | EVQLLESGGDLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPG<br>KGLEWVSAISGRGGTTYADSVKGRFTISRDNSKSTLCLQMNS<br>LRAEDTAVYYCAKARANWDYFDYWGQGT LTVSS     |
| SEQ ID No: 80 | VL 051 | DIQMTQSPSSVSASVGDRVITICRASQGISSWLAWYQHKPGK<br>APKLLIYAASILQSGVPSRFSGSGSGTDFTLTISSLRPEDFATYY<br>CQQANSFPITFGQGT RLEIK               |
| SEQ ID No: 81 | VH 055 | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMNWRQAPG<br>KGLEWVSAISGRGGTTYADSVKGRFTISRDNSKSTLCLQMNS<br>LRAEDTAVYYCAKARANWDYFDYWGQGT LTVSS      |
| SEQ ID No: 82 | VL 055 | DIQMTQSPSSVSASVGDRVITICRASQGISSWLAWYQHKPGK<br>APKLLIYAASILQSGVPSRFSGSGSGTDFTLTISSLRPEDFATYY<br>CQQANSFPITFGQGT RLEIK               |
| SEQ ID No: 83 | VH 123 | QVQLVQSGAEVKKPGASVKVSCKAAGYTFTNYGISWVRQAPG<br>QALEWMGWITTYSSNTIYAQKLQGRVTMTTDTSTSTAYMELRS<br>LRSDDTAVYYCARDRVVVRPDYFDYWGQGT LTVSS  |
| SEQ ID No: 84 | VL 123 | EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP<br>RLLIYDTSNRATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCQ<br>QRSHWPRTFGQGT KVEIK                |
| SEQ ID No: 85 | VH 161 | QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPG<br>QGLEWMGWL SAYSGNTIYAQKLQGRVTMTTDTSTTTAYMELR<br>SLRSDDTAVYYCARDRIVVRPDYFDYWGQGT LTVSS |
| SEQ ID No: 86 | VL 161 | EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP<br>RLLIYDASN RATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCQ<br>QRSNWPRTFGQGT KVEIK               |
| SEQ ID No: 87 | VH 124 | QVQLVQSGAEVKKPGASVKVSCKAAGYTFTNYGISWVRQAPG<br>QGLEWMGWITTYNGNTIYAQRFQDRVMTTDTSTSTAYMELRS<br>LRSDDTAVYYCARDRIIVRPDYFDYWGQGT LTVSS   |
| SEQ ID No: 88 | VL 124 | EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQQKPGQAP<br>RLLIYDASN RATGIPARFSGSGSGTDFTLTISLEPEDFAVYYCQ<br>QRSNWPRTFGQGT KVEIK               |

|                |        |                                                                                                                                   |
|----------------|--------|-----------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No: 89  | VH 001 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWNWIRQPPG<br>KGLEWIGEINHSGSTNYPNPSLKSRTISVDTSKNQFSLKLSSVT<br>AADTAVYYCARGNYGSGYYYFDLWGRGTQVTVSS  |
| SEQ ID No: 90  | VL 001 | DIQMTQSPSSLSASVGDRVITICRASQGISSWLAWYQQKPEK<br>APKSLIFAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYISFPITFGQGTREIK                 |
| SEQ ID No: 91  | VH 143 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWNWIRQPPG<br>KGLEWIGEIIHSGSANYNPSLMSRTISVDTSKNQFSLQLSSV<br>TAADTAVYYCARGYYGSGYYYFDYWGGGTLTVSS    |
| SEQ ID No: 92  | VL 143 | DIQMTQSPSSLSASVGDRVITICRASQGISSWLAWYQQKPEK<br>APKSLIYAASRLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPITFGQGTREIK                 |
| SEQ ID No: 93  | VH 019 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPG<br>KGLEWIGEIIHVGSTNYPNPSLKSRTISVDTSKSQFSLKLSSVT<br>AADTAVYYCARGYYDSGVYYFDYWAQGTTLTVSS  |
| SEQ ID No: 94  | VL 019 | DIQMTQSPSSLSASVGDRVITICRASQGISRWLAWYQQKPEK<br>APKSLIYAASSLRSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPITFGQGTREIK                 |
| SEQ ID No: 95  | VH 021 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPG<br>KGLEWIGEIIHSGSTNYPNPSLKSRTISVDTSKNQFSLKLSSVT<br>AADTAVYYCARGYYASGVYYFDYWGQGTTLTVSS  |
| SEQ ID No: 96  | VL 021 | DIQMTQSPSSLSASVGDRVITICRASQGISSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPITFGQGTREIK                 |
| SEQ ID No: 97  | VH 027 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYFWNWIRQPPG<br>KGLEWIGEIIHSGSTNYPNPSLKSRTISVDTSKNQFSLNLSSVT<br>AADTAVYYCARGLIGSGYYYFDYWDQGTTLTVSS  |
| SEQ ID No: 98  | VL 027 | DIQMTQSPSSLSASVGDRVITICRASQGISSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPITFGQGTREIK                 |
| SEQ ID No: 99  | VH 032 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPG<br>KGLEWIGEINHSGDTNYPNPSLTSRTISVDTSKNQFSLKLSSVT<br>AADTAVYYCARLFYGSIGIYYFDYWGQGTTLTVSS |
| SEQ ID No: 100 | VL 032 | DIQMTQSPSSLSASVGDRVITICRASQGISSWLAWYQQKPEK<br>APKSLIYATFRLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSFPPTFGQGTKVEIK                |
| SEQ ID No: 101 | VH 035 | QVQLQQWGAGLLKPSETLSLTCAIYGGSFSGYYWSWIRQPPG<br>KGLEWIGEINHSGDTNYPNPSLTSRTISVDTSKNQFSLKLSSVT<br>AADTAVYYCARLFYGSIGIYYFDYWGQGTTLTVSS |
| SEQ ID No: 102 | VL 035 | DIQMTQSPSSLSASVGDRVITICRASQGISSWLAWYQQKPEK<br>APKSLIYATFRLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSFPPTFGQGTKVEIK                |
| SEQ ID No: 103 | VH 036 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWSWIRQPPG<br>KGLEWIGEINHSGSTNYPNPSLKSRTISVDTSKNQFSLKLSSVT<br>AADTAVYYCARLYYGSPTYFDYWGQGTTLTVSS   |
| SEQ ID No: 104 | VL 036 | DIQMTQSPSSLSASVGDRVITICRASQGISSWLTWYQQKPEKA<br>PKSLIYAASRLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYC<br>QQYNSFPPTFGQGTKVEIK                |
| SEQ ID No: 105 | VH 054 | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPG<br>KGLEWIGEIIHSGSTNYPNPSLKSRTISVDTSKNQFSLKLSSVT<br>AADTAVYYCARLWYGSYSYFDYWGQGTTLTVSS   |
| SEQ ID No: 106 | VL 054 | DIQMTQSPSSLSASVGDRVITICRASQGISSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSFPPTFGGGTKVEIK                |

|                |        |                                                                                                                                    |
|----------------|--------|------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No: 107 | VH 094 | QVQLQQWGAGLLKPSETLSLTCAVSGGSFSGYYWTWIRQPPG<br>KGLEWIGEIIYHSGDTNYPNPSLKSRTISVDTSKNQFSLKLYSVT<br>AADTAVYYCARLYFGSGIYYLDYWGQGTLVTVSS  |
| SEQ ID No: 108 | VL 094 | DIQMTQSPSSLSASVGDRVITICRASQGIISSWLWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSFPPTFGQGTKVEIK                 |
| SEQ ID No: 109 | VH 105 | EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMSWVRQAPG<br>KGLEWVSAISGSAYSTYYADSVKGRFTISRDNKNTLWLQMNS<br>LRAEDTAVYYCAKAHYHGSGSYTLFDYWGQGTLVTVSS |
| SEQ ID No: 110 | VL 105 | DIQMTQSPSSLSASVGDRVITICRASQGIISSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPYTFGQGTKLEIK                |
| SEQ ID No: 111 | VH 100 | EVQLLESGGGLVQPGGSLRLSCAASGFTFNNGMNWVRQAPG<br>KGLEWVSAISGTGYSTYYADSVKGRFTISRDNKNTLYLQMNS<br>LRAEDTAVYYCAKAHYFGSGSYTLFDYWGQGTLVTVSS  |
| SEQ ID No: 112 | VL 100 | DIQMTQSPSSLSASVGDRVITICRASQGIISSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPYTFGQGTKLEIK                |
| SEQ ID No: 113 | VH 125 | EVQLLESGGGLVQPGGSLRLSCAASGFTFTDYAMNWRQAPG<br>KGLEWVSTISGSGYATYYADSVKGRFTISRDNKNTLYLQMNS<br>LRAEDTAVYYCAKGHTLGSGSYTLFDYWGQGTLVTVSS  |
| SEQ ID No: 114 | VL 125 | DIQMTQSPSSLSASVGDRVITICRASQGINSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPYTFGQGTKLEIK                 |
| SEQ ID No: 115 | VH 162 | EVQLWESGGGSVQPGGSLRLSCAASGFTFSSYGMSWVRQAP<br>GKLEWVSGISGSGYSTYYADSVKGRFTISRDNKNTLYLQMN<br>SLRAEDTAVYYCAKGYYHGSGSYTTFDYWGQGTLVTVSS  |
| SEQ ID No: 116 | VL 162 | DIQMTQSPSSLSASVGDRVITICRASQGIISSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPLTFGGGTKVEIK                |
| SEQ ID No: 117 | VH 033 | QVQLVESGGGVVQTGRSLRLSCAASGFTFSSHAMHWVRQAPG<br>KGLEWVAAISYDGSNKYYADSVKGRFTISRDNKNTLYLQMNS<br>LRAEDTAVYYCARGDYISSSGVFDYWGQGTLVTVSS   |
| SEQ ID No: 118 | VL 033 | DIQMTQSPSSLSASVGDRVITICRASQGIISSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPITFGQGTRLEIK                |
| SEQ ID No: 119 | VH 160 | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSHAMHWVRQAPG<br>KGLEWVAAISYDGSNKYYADSVKGRFTISRDNKNTMYLQMN<br>SLRAEDTAMCYCARGSITGSTGVFDYWGQGTLVTVSS   |
| SEQ ID No: 120 | VL 160 | DIQMTQSPSSLSASVGDRVITICRASQDISSWLAWYQQKPEK<br>APKSLIYAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPITFGQGTRLEIK                 |
| SEQ ID No: 121 | VH 166 | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPG<br>KGLEWVAVISYDGSNEYADSVKGRFTISRDNKNTLYLQMNS<br>LRAEDTAVYYCARGSIIGSTGVFDYWGQGTLVTVSS    |
| SEQ ID No: 122 | VL 166 | DIQMTQSPSSLSASVGDRVITICRASQGINWLAWYQQKPEK<br>APKSLIYDASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQYNSYPITFGQGTRLEIK                  |
| SEQ ID No: 123 | VH 152 | QVQVVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPG<br>KGLEWVAVISYDGSYKYYADSVKGRFTISRDNKNTLYLQMNS<br>LRAEDTAVYYCARGSITGSTGVFDYWGQGTLVTVSS   |
| SEQ ID No: 124 | VL 152 | DIQMTQSPSSLSASVGDRVITICRASQGINSWLAWYQQKPEK<br>APKSLIYDASSLQSGVPSRFSGSGSGTDFTLTISLQPENFATYY<br>CQQYNSYPITFGQGTRLEIK                 |

|                |        |                                                                                                                                     |
|----------------|--------|-------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No: 125 | VH 167 | QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAIHWVRQAPG<br>KGLEWVAVISYDGSNKYYADSVKGRFTISRDN SKNTLYLQMNS<br>LRAEDTAVYYCARG SITGSTGVFDYWGQGTLVTVSS |
| SEQ ID No: 126 | VL 167 | DIQMTQSPSSLSASVGDRVTITCRASQGISNWLAWYQQKPEK<br>APKSLIYDASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYY<br>CQQNSYPITFGQGTRLEIK                   |

**Table 4:** Consensus CDRs based on sequence alignments shown in Figures 1 and 2.

|                                       |             |            |                     |                                                                                                                         |
|---------------------------------------|-------------|------------|---------------------|-------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No: 9<br>050-049-051-<br>055   | IgHV3-23-1  | VH<br>CDR1 | GFTFSSYA            |                                                                                                                         |
| SEQ ID No: 127<br>050-049-051-<br>055 | IgHV3-23-1  | VH<br>CDR2 | ISGX1GGX2T          | Wherein X1=R or S, and<br>X2=T or S; preferably,<br>wherein X1=R and X2=T                                               |
| SEQ ID No: 11<br>050-049-051-<br>055  | IgHV3-23-1  | VH<br>CDR3 | AKARANWDYFD<br>Y    |                                                                                                                         |
| SEQ ID No: 128<br>084                 | IgHV1-69-04 | VH<br>CDR1 | GGTFX1X2YA          | Wherein X1=R or S, and<br>X2=T or S; preferably,<br>wherein X1=R and X2=T                                               |
| SEQ ID No: 129<br>084                 | IgHV1-69-04 | VH<br>CDR2 | IX2X3X3LGIX4        | Wherein X1=N or I, X2=T or<br>P, X3=V or I, and X4=V or A,<br>preferably, wherein X1=N,<br>X2=T, X3=V, and X4=V         |
| SEQ ID No: 130<br>084                 | IgHV1-69-04 | VH<br>CDR3 | AREKGV DYYG<br>X1X2 | Wherein X1=I or M, X2=E or<br>D; preferably, wherein X1=I,<br>X2=E                                                      |
| SEQ ID No: 131<br>169-123-161-<br>124 | IgHV1-18-1  | VH<br>CDR1 | GYTFTXYG            | Wherein X=N or S, preferably<br>N                                                                                       |
| SEQ ID No: 132<br>169-123-161-<br>124 | IgHV1-18-1  | VH<br>CDR2 | X1X2X3YX4X5<br>GNT  | Wherein X1=L or I; X2=S, T,<br>or I; X3=A or T; X4=S or N;<br>X5=G or S; preferably,<br>wherein X2=S, X3=A, and<br>X4=S |

|                                               |             |            |                         |                                                                                                                                           |
|-----------------------------------------------|-------------|------------|-------------------------|-------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No: 133<br>169-123-161-<br>124         | IgHV1-18-1  | VH<br>CDR3 | ARDRX1X2VRP<br>DYFDY    | Wherein X1=I or V, X2=V or<br>I; preferably, wherein X1=I<br>and X2=V                                                                     |
| SEQ ID No: 134<br>025-001-143-<br>019-021-027 | IgHV4-34-01 | VH<br>CDR1 | GGSF SX1YX2             | Wherein X1=D or G and<br>X2=Y or F; preferably,<br>wherein X1=D and X2=Y                                                                  |
| SEQ ID No: 135<br>025-001-143-<br>019-021-027 | IgHV4-34-01 | VH<br>CDR2 | IX1HX2GSX3              | Wherein X1=H or N, X2=S or<br>V, and X3=T or A; preferably,<br>wherein X1=H, X2=S, and<br>X3=T                                            |
| SEQ ID No:136<br>025-001-143-<br>019-021-027  | IgHV4-34-01 | VH<br>CDR3 | ARGX1X2X3SG<br>X4YYFDX5 | Wherein X1=Y, N or L; X2=Y<br>or I, X3=D, G or A; X4=V or<br>Y; and X5=Y or L; preferably,<br>wherein X1=Y, X2=Y, X3=D,<br>X4=V, and X5=Y |
| SEQ ID No:137<br>091-032-035-<br>036-054-094  | IgHV4-34-01 | VH<br>CDR1 | GGSF SX1YY              | Wherein X1=G or D,<br>preferably G                                                                                                        |
| SEQ ID No:138<br>091-032-035-<br>036-054-094  | IgHV4-34-01 | VH<br>CDR2 | IX1HSGX2T               | Wherein X1=Y, N or H; and<br>X2=D or S; preferably,<br>wherein X1=Y and X2=D                                                              |
| SEQ ID No:139<br>091-032-035-<br>036-054-094  | IgHV4-34-01 | VH<br>CDR3 | ARLX1X2GSGX<br>3YYX4DY  | Wherein X1=Y, F or W; X2=F<br>or Y; X3=I, T or S; and X4=L<br>or F; preferably, wherein<br>X1=Y, X2=F, X3=I, and X4=L                     |
| SEQ ID No:140<br>129                          | IgHV3-30-01 | VH<br>CDR1 | GFTFSX1X2A              | Wherein X1=T or F, X2=F or<br>Y; preferably, wherein X1=T<br>and X2=F                                                                     |
| SEQ ID No:141<br>129                          | IgHV3-30-01 | VH<br>CDR2 | ISYDGX1X2K              | Wherein X1=G or S, X2=H or<br>N; preferably, wherein X1=G<br>and X2=H                                                                     |
| SEQ ID No:142<br>129                          | IgHV3-30-01 | VH<br>CDR3 | ARGLGVWGX1F<br>DY       | Wherein X1=A or Y,<br>preferably A                                                                                                        |

|                                              |                    |            |                           |                                                                                                                                |
|----------------------------------------------|--------------------|------------|---------------------------|--------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No:143<br>098-105-100-<br>125-162     | IgHV3-23-01        | VH<br>CDR1 | GFTFX1X2YX3               | Wherein X1=S, N or T; X2=N,<br>D or S; and X3=G or A;<br>preferably, wherein X1=S,<br>X2=N and X3=G                            |
| SEQ ID No:144<br>098-105-100-<br>125-162     | IgHV3-23-01        | VH<br>CDR2 | ISGX1X2X3X4T              | Wherein X1=S or T, X2=A or<br>G, X3=Y or G, X4=S or A;<br>preferably, wherein X1=S,<br>X2=A, X3=Y, X4=S                        |
| SEQ ID No:145<br>098-105-100-<br>125-162     | IgHV3-23-01        | VH<br>CDR3 | AKX1X2X3X4G<br>SGSYTX5FDY | Wherein X1=A or G; X2=H or<br>Y; X3=Y or T; X4=H, F or L;<br>X5=L or S; preferably,<br>wherein X1=A; X2=H; X3=Y;<br>X4=H; X5=L |
| SEQ ID No:146<br>127                         | IgHV5-51-01        | VH<br>CDR1 | GYSFX1X2YW                | Wherein X1=S or T, X2=I or<br>S; preferably, wherein X1=S,<br>X2=I                                                             |
| SEQ ID No:147<br>127                         | IgHV5-51-01        | VH<br>CDR2 | IX1PGDSDX2                | Wherein X1=F or Y, X2=I or<br>T; preferably, wherein X1=F,<br>X2=I                                                             |
| SEQ ID No:148<br>127                         | IgHV5-51-01        | VH<br>CDR3 | ARQPGDWSPR<br>HWYFDL      |                                                                                                                                |
| SEQ ID No:149<br>159                         | IgHV5-51-01        | VH<br>CDR1 | GYXFTSYW                  | Wherein X=N or S, preferably<br>N                                                                                              |
| SEQ ID No:51<br>159                          | IgHV5-51-01        | VH<br>CDR2 | IYPGDSDT                  |                                                                                                                                |
| SEQ ID No:52<br>159                          | IgHV5-51-01        | VH<br>CDR3 | ARWGTYDILT<br>GYFN        |                                                                                                                                |
| SEQ ID No:71<br>132                          | IgHV1-18-01        | VH<br>CDR1 | GYTFTSYG                  |                                                                                                                                |
| SEQ ID No:150<br>132                         | IgHV1-18-01        | VH<br>CDR2 | ISAYNGNX                  | Wherein X=S or T, preferably<br>S                                                                                              |
| SEQ ID No:151<br>132                         | IgHV1-18-01        | VH<br>CDR3 | AREYSYDSGT<br>FYYGMDV     |                                                                                                                                |
| SEQ ID No:152<br>153-033-160-<br>166-152-167 | IgHV3-30-<br>03-01 | VH<br>CDR1 | GFTFSX1X2X3               | Wherein X1=D or S, X2=Y or<br>H, X3=V or A; preferably,<br>wherein X1=D, X2=Y, X3=V                                            |

|                                              |                    |            |                           |                                                                                                                                                                         |
|----------------------------------------------|--------------------|------------|---------------------------|-------------------------------------------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID No:153<br>153-033-160-<br>166-152-167 | IgHV3-30-<br>03-01 | VH<br>CDR2 | ISYDGSX1X2                | Wherein X1=N or Y, X2=K or<br>E, preferably wherein X1=N<br>and X2=K                                                                                                    |
| SEQ ID No:154<br>153-033-160-<br>166-152-167 | IgHV3-30-<br>03-01 | VH<br>CDR3 | ARGX1X2X3X4<br>X5X6GX7FDY | Wherein X1=G, D or S; X2=I<br>or Y; X3=T or I; X4=G or S;<br>X5=T or S; X6=T or S; X7=Y<br>or V; preferably, wherein<br>X1=G; X2=I; X3=T; X4=G;<br>X5=T; X6=T; and X7=V |
| SEQ ID No:13<br>050-084-049-<br>051-055      | IgKV1-12-01        | VL<br>CDR1 | QGISSW                    |                                                                                                                                                                         |
| 050-084-049-<br>051-055                      | IgKV1-12-01        | VL<br>CDR2 | XAS                       | Wherein X=A or V                                                                                                                                                        |
| SEQ ID No:155<br>050-084-049-<br>051-055     | IgKV1-12-01        | VL<br>CDR3 | QQANSFPXT                 | Wherein X=I or L                                                                                                                                                        |
| SEQ ID No:6<br>169-124-161-<br>123           | IgKV3-11-01        | VL<br>CDR1 | QSVSSY                    |                                                                                                                                                                         |
| 169-124-161-<br>123                          | IgKV3-11-01        | VL<br>CDR2 | DXS                       | Wherein X=A or T, preferably<br>A                                                                                                                                       |
| SEQ ID No:156<br>169-124-161-<br>123         | IgKV3-11-01        | VL<br>CDR3 | QQRXSWPRT                 | Wherein X=N or H, preferably<br>N                                                                                                                                       |
| SEQ ID No:157<br>025-001-019-<br>143-021-027 | IgKV1D-16-<br>01   | VL<br>CDR1 | QGISXW                    | Wherein X=R or S, preferably<br>R                                                                                                                                       |
| 025-001-019-<br>143-021-027                  | IgKV1D-16-<br>01   | VL<br>CDR2 | AAS                       |                                                                                                                                                                         |
| SEQ ID No:164<br>025-001-019-<br>143-021-027 | IgKV1D-16-<br>01   | VL<br>CDR3 | QQYNSXPIT                 | Wherein X=Y or F, preferably<br>Y                                                                                                                                       |
| SEQ ID No:33<br>091-032-035-<br>036-054-094  | IgKV1D-16-<br>01   | VL<br>CDR1 | QGISSW                    |                                                                                                                                                                         |

|                                          |              |            |           |                                                           |
|------------------------------------------|--------------|------------|-----------|-----------------------------------------------------------|
| 091-032-035-036-054-094                  | IgKV1D-16-01 | VL<br>CDR3 | QQYNSFPPT |                                                           |
| SEQ ID No:159<br>098-100-105-125-162     | IgKV1D-16-01 | VL<br>CDR1 | QGIXSW    | Wherein X=S or N, preferably S                            |
| 098-100-105-125-162                      | IgKV1D-16-01 | VL<br>CDR2 | AAS       |                                                           |
| SEQ ID No:160<br>098-100-105-125-162     | IgKV1D-16-01 | VL<br>CDR3 | QQYNSYPXT | Wherein X=Y or L, preferably Y                            |
| SEQ ID No:161<br>153-152-166-167-160-033 | IgKV1D-16-01 | VL<br>CDR1 | QGIX1X2W  | Wherein X1=S or N; X2=S or N; preferably, wherein X1=X2=S |
| 153-152-166-167-160-033                  | IgKV1D-16-01 | VL<br>CDR2 | XAS       | Wherein X=D or A, preferably D                            |
| SEQ ID No:162<br>153-152-166-167-160-033 | IgKV1D-16-01 | VL<br>CDR3 | QQYXSYPT  | Wherein X=K or N, preferably K                            |

#### Example 11 - Purification of antibodies

Culture supernatant was filtered over 0.2 µm dead-end filters, loaded on 5 ml MabSelect SuRe columns (GE Health Care) and eluted with 0.1 M sodium citrate-NaOH, pH 3. The eluate was immediately neutralized with 2M Tris-HCl, pH 9 and dialyzed overnight to 12.6 mM NaH<sub>2</sub>PO<sub>4</sub>, 140 mM NaCl, pH 7.4 (B.Braun). Alternatively, subsequent to purification, the eluate was loaded on a HiPrep™ Desalting column and the antibody was exchanged into 12.6 mM NaH<sub>2</sub>PO<sub>4</sub>, 140 mM NaCl, pH 7.4 (B.Braun) buffer. After dialysis or exchange of buffer, samples were sterile filtered over 0.2 µm dead-end filters. Purity was determined by SDS-PAGE and concentration was measured by nephelometry and absorbance at 280 nm. Purified antibodies were stored at 4°C. Mass spectrometry was performed to identify the molecular mass of the antibody heavy and light chains expressed by the hybridomas as described in Example 9.

### **Example 12 – Binding of HER2 clones to tumor cells expressing membrane-bound HER2 measured by means of FACS analysis**

The binding of HER2 antibodies to AU565 cells (purchased at ATCC, CRL-2351) and A431 cells (purchased at ATCC, CRL-1555), was tested using flow cytometry (FACS Canto II, BD Biosciences). Qif analysis (Dako, Glostrup, Denmark) revealed that AU565 cells expressed on average 1,000,000 copies of HER2 protein per cell, whereas A431 cells expressed on average 15,000 copies per cell. Binding of HER2 antibodies was detected using a Phycoerythrin (PE)-conjugated goat-anti-human IgG antibody (Jackson). Trastuzumab (clinical-grade Herceptin®) was used as positive control antibody, and an isotype control antibody was used as negative control antibody. EC<sub>50</sub> values were determined by means of non-linear regression (sigmoidal dose-response with variable slope) using GraphPad Prism V4.03 software (GraphPad Software, San Diego, CA, USA).

As shown in Figure 3, all tested HER2 antibodies bound to HER2 expressed on both AU565 and A431 cells in a dose-dependent manner. The EC<sub>50</sub> values for binding varied between 0.336-2.290 µg/mL for AU565 cells and 0.068-1.135 µg/mL for A431 cells. Especially on A431 cells, large differences in EC<sub>50</sub> values were observed between the tested antibodies. However, antibody 098 had the best (i.e., lowest) EC<sub>50</sub> value on both types of cells. Also some differences in maximum binding levels were observed between different antibodies, on both AU565 and A431 cells. Of the tested antibodies, antibody 098 also had the highest maximum binding level on AU565 cells, whereas antibody 025 had the highest maximum binding level on A431 cells.

### **Example 13 - Binding of HER2 antibodies to membrane-bound HER2 expressed on Rhesus epithelial cells measured by means of FACS analysis**

To determine cross-reactivity with Rhesus HER2, the binding of HER2 antibodies to HER2-positive Rhesus epithelial cells (4MBr-5 purchased at ATCC) was tested using flow cytometry (FACS Canto II, BD Biosciences). A Phycoerythrin-conjugated goat-anti-human IgG antibody (Jackson) was used as a secondary conjugate. An isotype control antibody was used as negative control antibody.

As shown in Figure 4, all tested HER2 antibodies were cross-reactive with Rhesus monkey HER2. At both tested concentrations (1 µg/mL and 10 µg/mL), the HER2 antibodies were able to bind specifically to Rhesus monkey HER2. Antibody 127 demonstrated poor binding at 1 µg/mL concentration, but showed good binding at 10 µg/mL concentration. Antibody 098 had the highest binding level at both antibody concentrations. No binding was observed with the isotype control antibody.

**Example 14 - Competition of HER2 antibodies for binding to soluble Her2ECDHis measured in sandwich-ELISA**

The optimal coating concentrations of the tested HER2 antibodies and optimal Her2ECDHis concentration were determined in the following manner: ELISA wells were coated overnight at 4°C with HER2 HuMabs serially diluted in PBS (0.125-8 µg/mL in 2-fold dilutions). Next, the ELISA wells were washed with PBST (PBS supplemented with 0.05% Tween-20 [Sigma-Aldrich, Zwijndrecht, The Netherlands]) and blocked for one hour at room temperature (RT) with PBSTC (PBST supplemented 2% [v/v] chicken serum [Gibco, Paisley, Scotland]). The ELISA wells were then washed with PBST and incubated for one hour at RT with Her2ECDHis serially diluted in PBSTC (0.25-2 µg/mL in 2-fold dilutions). Unbound Her2ECDHis was washed away with PBST, and bound Her2ECDHis was incubated for one hour at RT with 0.25 µg/mL biotinylated rabbit-anti-6xhis-biot (Abcam, Cambridge, UK). The plate was thereafter washed with PBST and incubated for one hour with 0.1 µg/mL Streptavidin-poly-HRP (Sanquin, Amsterdam, The Netherlands) diluted in PBST. After washing, the reaction was visualized through a 15 minutes incubation with 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulfonic acid (ABTS: one ABTS tablet diluted in 50 mL ABTS buffer (Roche Diagnostics, Almere, The Netherlands)) at RT protected from light. The colorization was stopped by adding an equal volume of oxalic acid (Sigma-Aldrich, Zwijndrecht, The Netherlands). Fluorescence at 405 nm was measured on a microtiter plate reader (Biotek Instruments, Winooski, USA). The antibody concentrations that resulted in sub-optimal binding of each antibody were determined and used for the following cross-block experiments.

Each HER2 antibody was coated to the ELISA wells at the sub-optimal dose that was determined as described above. After blocking of the ELISA wells, the wells were incubated with the predetermined concentration of 1 µg/mL biotinylated Her2ECDHis in the presence or absence of an excess of a second (competitor) HER2 antibody. The ELISA was then performed as described above. Residual binding of Her2ECDHis to the coated antibody was expressed as a percentage relative to the binding observed in the absence of competitor antibody. Percentage competition was then determined as 100 minus the percentage of inhibition. 75% competition was considered as complete cross-block, whereas 25-74% competition was considered as partial cross-block, and 0-24% competition was considered non-blocking.

As shown in Table 5, all HER2 antibodies were found to be able to block binding to Her2ECDHis, at least partially, for themselves. After dividing the antibodies into 3 major cross-block groups, all antibodies were tested for competition with at least one representative antibody from each group.

The first group comprised trastuzumab and antibodies 169, 050 and 084, which blocked each other for binding to Her2ECDHis, but did not cross-block antibodies from other groups.

The second group comprised pertuzumab and antibodies 025, 091 and 129, which blocked each other for binding to Her2ECDHis, except for antibodies 129 and 091 which both cross-blocked pertuzumab and 025, but not each other. None of the antibodies of group 2 blocked antibodies from other groups.

A third group comprised antibodies C1, F5, 127, 098, 132, 153 and 159, which did not cross-block any antibody from the other groups. Within this group 3, some variation was observed. Antibody 127 was the only antibody that was able to cross-block all other antibodies in this group for binding to Her2ECDHis; antibody 159 cross-blocked all other antibodies within this group, except 132; clone 098 cross-blocked all antibodies of group 3, except 132 and 153; antibody 153 cross-blocked 127, 132 and 159 for binding to Her2ECDHis, but not 098, C1 or F5; clone 132 cross-blocked 127, 132 and 153. When added as competitor antibodies, F5 and C1 only demonstrated cross-blocking of each other. However, the reverse reaction also revealed competition with antibodies 127, 098 and 159, but not 153 and 132. Possibly, these differences may have resulted from lower affinities of antibodies C1 and F5 for Her2ECDHis.

Values higher than 100% can be explained by avidity effects and the formation of antibody-Her2ECDHis complexes containing two non-competing antibodies.

**Table 5:** Competition and cross-blocking of HER2 antibodies for binding to Her2ECDHis

| Immobilized mAb ↓ | Competing mAb → |     |     |     |      |     |     |     |     |     |     |     |     |     |     |
|-------------------|-----------------|-----|-----|-----|------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
|                   | tras            | 169 | 050 | 084 | pert | 025 | 091 | 129 | C1  | F5  | 127 | 159 | 098 | 153 | 132 |
| Trastuzumab       | 6               | 15  | 6   | 51  | 100  | 107 | 100 | 85  | 103 | 99  | 115 | 90  | 101 | 101 | 101 |
| TH1014-169        | 12              | 45  | 21  | 73  | 101  | 98  | 105 | 106 | ND  | ND  | ND  | ND  | 105 | 102 | ND  |
| TH1014-050        | 12              | 30  | 12  | 74  | 95   | 104 | 98  | 110 | ND  | ND  | ND  | ND  | 102 | 104 | ND  |
| TH1014-084        | 74              | 73  | 76  | 26  | 101  | 106 | 104 | 104 | ND  | ND  | ND  | ND  | 109 | 98  | ND  |
| TH1014-pert       | 104             | 100 | 94  | 95  | 9    | 20  | 19  | 39  | 106 | 125 | 116 | 81  | 103 | 100 | 109 |
| TH1014-025        | 98              | 98  | 100 | 104 | 6    | 13  | 21  | 15  | ND  | ND  | ND  | ND  | 102 | 99  | ND  |
| TH1014-091        | 99              | 99  | 95  | 100 | 6    | 13  | 15  | 78  | ND  | ND  | ND  | ND  | 98  | 98  | ND  |
| TH1014-129        | 93              | 99  | 97  | 92  | 22   | 55  | 76  | 12  | ND  | ND  | ND  | ND  | 106 | 98  | ND  |
| TH1014-C1         | 89              | ND  | ND  | ND  | ND   | ND  | ND  | ND  | 65  | 58  | 73  | 53  | 58  | 77  | 90  |
| TH1014-F5         | 197             | ND  | ND  | ND  | ND   | ND  | ND  | ND  | 70  | 21  | 62  | 15  | 16  | 80  | 125 |
| TH1014-127        | 102             | ND  | ND  | ND  | ND   | ND  | ND  | ND  | 112 | 88  | 11  | 3   | 58  | 21  | 44  |
| TH1014-159        | 111             | ND  | ND  | ND  | 112  | ND  | ND  | ND  | 96  | 86  | 15  | 6   | 11  | 40  | 79  |

|                   |     |     |     |     |     |     |     |     |     |     |    |     |     |     |     |
|-------------------|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|----|-----|-----|-----|-----|
| TH1014-098        | 107 | 102 | 100 | 103 | 104 | 108 | 104 | 107 | 125 | 96  | 21 | 9   | 17  | 110 | 142 |
| TH1014-153        | 134 | 111 | 103 | 107 | 121 | 97  | 102 | 106 | 257 | 96  | 27 | 23  | 115 | 28  | 33  |
| TH1014-132        | 353 | ND  | ND  | ND  | 288 | ND  | ND  | ND  | 422 | 379 | 30 | 131 | 309 | 41  | 32  |
| Cross-block group | 1   | 1   | 1   | 1   | 2   | 2   | 2   | 2b  | 3a  | 3a  | 3a | 3a  | 3a  | 3b  | 3b  |

75 – 100% competition

25 – 74% competition

0 – 24% competition

Depicted values are mean percentages of binding relative to the binding observed in the absence of competitor antibody, of two independent experiments. Competition experiments with HEK produced TH1014-C1 and TH1014-F5 were performed once. Trastuzumab (clinical grade Herceptin®) and HEK-produced pertuzumab (TH1014-pert) were also tested.

#### Example 15 - Antibody-dependent cell-mediated cytotoxicity (ADCC)

SK-BR-3 cells (purchased at ATCC, HTB-30) were harvested ( $5 \times 10^6$  cells), washed (twice in PBS, 1500 rpm, 5 min) and collected in 1 mL RPMI 1640 medium supplemented with 10% cosmic calf serum (CCS) (HyClone, Logan, UT, USA), to which 200  $\mu\text{Ci}$   $^{51}\text{Cr}$  (Chromium-51; Amersham Biosciences Europe GmbH, Roosendaal, The Netherlands) was added. The mixture was incubated in a shaking water bath for 1.5 hours at 37°C. After washing of the cells (twice in PBS, 1500 rpm, 5 min), the cells were resuspended in RPMI 1640 medium supplemented with 10% CCS, counted by trypan blue exclusion and diluted to a concentration of  $1 \times 10^5$  cells/mL.

Meanwhile, peripheral blood mononuclear cells (PBMCs) were isolated from fresh buffy coats (Sanquin, Amsterdam, The Netherlands) using standard Ficoll™ density centrifugation according to the manufacturer's instructions (lymphocyte separation medium; Lonza, Verviers, France). After resuspension of cells in RPMI 1640 medium supplemented with 10% CCS, cells were counted by trypan blue exclusion and concentrated to  $1 \times 10^7$  cells/mL.

Trastuzumab was produced in CHO cells resulting in an (increased) non-core fucosylation grade of 12.4%, whereas the other HER2 antibodies were produced in HEK cells, resulting on average in 4% non-core fucosylation.

For the ADCC experiment, 50  $\mu\text{L}$   $^{51}\text{Cr}$ -labeled SK-BR-3 cells (5,000 cells) were pre-incubated with 15  $\mu\text{g/mL}$  HER2 antibody (IgG1, $\kappa$ ) in a total volume of 100  $\mu\text{L}$  RPMI medium supplemented with 10% CCS in a 96-well microtiter plate. After 15 min at RT, 50  $\mu\text{L}$  PBMCs (500,000 cells) were added, resulting in an effector to target ratio of 100:1. The maximum

amount of cell lysis was determined by incubating 50  $\mu$ L  $^{51}\text{Cr}$ -labeled SK-BR-3 cells (5000 cells) with 100  $\mu$ L 5% Triton-X100™. The amount of spontaneous lysis was determined by incubating 5000  $^{51}\text{Cr}$ -labeled SK-BR-3 cells in 150  $\mu$ L medium, without any antibody or effector cells. The level of antibody-independent cell lysis was determined by incubating 5000 SK-BR-3 cells with 500,000 PBMCs without antibody. Subsequently, the cells were incubated 4 hr at 37°C, 5% CO<sub>2</sub>. To determine the amount of cell lysis, the cells were centrifuged (1200 rpm, 3 min) and 75  $\mu$ L of supernatant was transferred to micronic tubes, after which the released  $^{51}\text{Cr}$  was counted using a gamma counter. The measured counts per minute (cpm) were used to calculate the percentage of antibody-mediated lysis as follows:

$$(cpm \text{ sample} - cpm \text{ Ab-independent lysis}) / (cpm \text{ max. lysis} - cpm \text{ spontaneous lysis}) \times 100\%$$

As shown in Figure 5, HER2 antibodies from cross-block groups 1 and 2 induced efficient lysis of SK-BR-3 cells through ADCC. From group 3, antibody 153 was the only antibody that induced efficient ADCC, antibody 132 induced about 10% ADCC, and clones 098, 159 and 127 did not induce ADCC.

#### **Example 16 - Inhibition of ligand-independent proliferation of AU565 cells**

HER2 antibodies were tested for their ability to inhibit proliferation of AU565 cells *in vitro*. Due to the high HER2 expression levels on AU565 cells (~1,000,000 copies per cell as described in Example 12), HER2 is constitutively active in these cells and thus not dependent on ligand-induced heterodimerization.

In a 96-well tissue culture plate (Greiner bio-one, Frickenhausen, Germany), 9000 AU565 cells were seeded per well in the presence of 10  $\mu$ g/mL HER2 antibody in serum-free cell culture medium. As a control, cells were seeded in serum-free medium without antibody. After 3 days, the amount of viable cells was quantified with Alamarblue™ (BioSource International, San Francisco, US) according to the manufacturer's instructions. Fluorescence was monitored using the EnVision™ 2101 Multilabel reader (PerkinElmer, Turku, Finland) with standard Alamarblue™ settings. The Alamarblue™ signal of antibody-treated cells was plotted as a percentage relative to untreated cells. Dunnett's test was applied for statistical analysis.

The results are shown in Figure 6, depicting the percentage proliferation of AU565 cells after HER2 antibody treatment compared to untreated cells, which was set to 100%. Of the tested Group 1 antibodies, trastuzumab, 050 and 169 demonstrated significant inhibition of AU565 cell proliferation ( $P < 0.05$ ), whereas 084 had no effect. None of the tested antibodies from group 2 (Pertuzumab, 025, 092 and 129) was able to inhibit AU565

cell proliferation. The tested antibodies from group 3 (098 and 153) did not inhibit AU565 proliferation. In contrast, both antibodies induced enhanced proliferation of AU565 cells compared to untreated cells (098 more than 153). Enhancing proliferation can be an advantage in some therapeutic applications of ADC-conjugates, e.g., where the cytotoxic action of the drug relies on, or is enhanced by, cell proliferation. For trastuzumab and pertuzumab, this was in accordance with the results described by Juntilla *et al.* (Cancer Cell 2009;15(5):353–355).

#### **Example 17 - Inhibition of ligand-induced proliferation of MCF-7 cells**

Since HER2 is an orphan receptor, its signaling is mainly dependent on activation of other ErbB-family members such as EGFR and Her3. Upon ligand binding, these two receptors can bind to and activate the HER2 receptor, resulting in e.g. proliferation. Various publications describe that pertuzumab efficiently inhibits Heregulin- $\beta$ 1-induced proliferation (Franklin MC. Cancer Cell 2004 / Landgraf R. BCR 2007). For trastuzumab, it has been described that it has little effect on Heregulin- $\beta$ 1-induced HER2/HER3 heterodimerization and proliferation (Larsen SS., *et al.*, Breast Cancer Res Treat 2000;58:41-56; Agus DB., *et al.*, Cancer Cell 2002;2:127-137; Wehrman *et al.* (2006), *supra*).

To investigate the ability of the present human HER2 antibodies to interfere with Heregulin- $\beta$ 1-induced HER2/HER3 heterodimers, a Heregulin- $\beta$ 1-induced proliferation assay was performed. Therefore, MCF7 cells (purchased at ATCC, HTB-22) expressing ~20.000 HER2 molecules per cell, were seeded in a 96-wells tissue culture plate (Greiner bio-one) (2.500 cells/well) in complete cell culture medium. After 4 hours, the cell culture medium was replaced with starvation medium containing 1% Cosmic Calf Serum (CCS) and 10  $\mu$ g/mL HER2 antibody. Next, Heregulin- $\beta$ 1 (PeproTech, Princeton Business Park, US) diluted in 1% CCS containing starvation medium was added to the wells to a final concentration of 1.5 ng/ml. After 4 days incubation, the amount of viable cells was quantified with Alamarblue (BioSource International) according to the manufacturer's instructions. Fluorescence was monitored using the EnVision 2101 Multilabel reader (PerkinElmer) with standard Alamarblue settings. The Alamarblue signal of HER2 antibody-treated ligand-induced cells was plotted as a percentage signal compared to ligand-induced cells incubated without HER2 antibody. Dunnett's test was applied for statistical analysis.

Figure 7 shows the percentage of viable MCF7 cells stimulated with Heregulin- $\beta$ 1 and treated with the indicated HER2 antibody, relative to the viable cells after stimulation with Heregulin- $\beta$ 1 in the absence of HER2 antibody, which was set to 100%. MCF-7 proliferation in absence of both Heregulin- $\beta$ 1 and antibody was also depicted (none). Antibodies 025, 091, 129, 153 and pertuzumab (TH1014-pert) demonstrated significant inhibition of

Heregulin- $\beta$ 1-induced MCF-7 proliferation ( $P < 0.05$ ). Also trastuzumab showed some inhibition of Heregulin- $\beta$ 1-induced proliferation of MCF-7 cells, although not as efficient as the other tested HER2 antibodies. It has been reported that domain IV of HER2 is involved in the stabilization of EGFR/HER2 heterodimers, but without details on its contribution to HER2/HER3 heterodimers (Wehrman *et al.*, *supra*). Antibodies 050, 084, 169 and 098 had no statistically significant effect on Heregulin- $\beta$ 1-induced proliferation of MCF-7 cells. Without being limited to theory, this suggests that these antibodies do not inhibit ligand-induced HER2/HER3 heterodimerization.

#### **Example 18 - Anti-kappa-ETA' assay**

To investigate the suitability of HER2 antibodies for an antibody-drug conjugate approach, a generic *in vitro* cell-based killing assay using kappa-directed pseudomonas-exotoxin A (anti-kappa-ETA') was developed. The assay makes use of a high affinity anti-kappa domain antibody conjugated to a truncated form of the pseudomonas-exotoxin A. Upon internalization, the anti-kappa-ETA' domain antibody undergoes proteolysis and disulfide-bond reduction, separating the catalytic from the binding domain. The catalytic domain is transported from the Golgi to the endoplasmic reticulum via the KDEL retention motif, and subsequently translocated to the cytosol where it inhibits protein synthesis and induces apoptosis (ref. Kreitman RJ. *BioDrugs* 2009;23(1):1-13). In this assay, to identify HER2 antibodies that enable internalization and killing through the toxin, HER2 antibodies are pre-conjugated with the anti-kappa-ETA' before incubation with HER2-positive cells.

First, the optimal concentration of anti-kappa-ETA' was determined for each cell line, i.e. the maximally tolerated dose that does not lead to induction of non-specific cell death. AU565 cells (7500 cells/well) and A431 cells (2500 cells/well) were seeded in normal cell culture medium in 96-wells tissue culture plate (Greiner bio-one) and allowed to adhere for at least 4 hours. Next, cells were incubated with 100, 10, 1, 0.1, 0.01, 0.001 and 0  $\mu$ g/mL anti-kappa-ETA' dilutions in normal cell culture medium. After 3 days, the amount of viable cells was quantified with Alamarblue (BioSource International, San Francisco, US) according to the manufacturer's instruction. Fluorescence was monitored using the EnVision 2101 Multilabel reader (PerkinElmer, Turku, Finland) with standard Alamarblue settings. The highest concentration anti-kappa-ETA' that did not kill the cells by itself was used for following experiments (0.5  $\mu$ g/mL for AU565 and 1  $\mu$ g/mL for A431).

Next, antibody-mediated internalization and killing by the toxin was tested for different HER2 antibodies. Cells were seeded as described above. Dilution-series of HER2 antibodies were pre-incubated for 30 minutes with the predetermined concentration anti-kappa-ETA' before adding them to the cells. After 3 days of incubation, the amount of viable

cells was quantified as described above. The Alamarblue signal of cells treated with anti-kappa-ETA' conjugated antibodies was plotted compared to cells treated with antibody alone. 23.4 µg/mL Staurosporin was used as positive control for cell killing. An isotype control antibody was used as negative control.

As shown in Figure 8A,B and Table 6, all anti-kappa-ETA'-conjugated HER2 antibodies were able to kill AU565 cells in a dose-dependent manner. All tested anti-kappa-ETA'-conjugated HER2 antibodies demonstrated better killing of AU565 cells compared to both anti-kappa-ETA'-conjugated trastuzumab and anti-kappa-ETA'-conjugated pertuzumab (TH1014-pert). Moreover, the percentage of killed AU565 cells was higher for anti-kappa-ETA'-conjugated HER2 antibodies (70.3 – 49.9 %), compared to anti-kappa-ETA'-conjugated trastuzumab (31.9 %) and anti-kappa-ETA'-conjugated pertuzumab (47.51 %), and the EC<sub>50</sub> values were increased. EC<sub>50</sub> values for anti-kappa-ETA'-conjugated HER2 antibodies ranged between 12.12 ng/mL and 46.49 ng/mL) compared to 78.49 ng/mL for anti-kappa-ETA'-conjugated trastuzumab and 117.8 ng/mL for anti-kappa-ETA'-conjugated pertuzumab. Antibody 159 had the highest percentage of cell-kill, and 098 the lowest EC<sub>50</sub>.

**Table 6:** Data shown are EC<sub>50</sub> values and maximal percentage cell kill of AU565 cells treated with anti-kappa-ETA'-conjugated HER2 antibodies, measured in one representative experiment. Cell-kill induced by Staurosporin was set as 100% and MFI of untreated cells was set as 0%. Ndet = not detected.

| antibody        | % cells killed | EC50 ng/mL |
|-----------------|----------------|------------|
| PC1014-159      | 70.3           | 34.93      |
| PC1014-127      | 69.0           | 34.46      |
| PC1014-132      | 61.6           | 39.35      |
| PC1014-129      | 60.8           | 30.85      |
| PC1014-153      | 60.3           | 32.26      |
| PC1014-025      | 60.0           | 16.71      |
| PC1014-098      | 58.7           | 12.12      |
| PC1014-084      | 58.1           | 26.97      |
| PC1014-050      | 52.4           | 12.71      |
| PC1014-091      | 50.6           | 46.49      |
| PC1014-169      | 49.9           | 35.62      |
| TH1014-pert     | 47.5           | 117.8      |
| trastuzumab     | 31.9           | 78.49      |
| isotype control | Ndet           | Ndet       |

As shown in Figure 8C,D and Table 7, antibodies 025, 091, 098, 129 and 153 were able to induce effective killing of A431 cells (≥75%). The highest percentage of cell-kill, and lowest EC<sub>50</sub> was shown by antibody 098. When conjugated to anti-kappa-ETA', trastuzumab and

isotype control antibody did not induce killing of A431 cells. Antibodies 169, 084 and pertuzumab induced percentages of cell kill of no more than about 50%. No cell kill was observed with non-conjugated HER2 antibodies.

**Table 7:** Data shown are EC<sub>50</sub> values and maximal percentage cell kill of A431 cells treated with anti-kappa-ETA'-conjugated HER2 antibodies, measured in one representative experiment. Cell kill induced by Staurosporin was set as 100% and MFI of untreated cells was set as 0%. "NDet" means not detected.

| antibody        | % cells killed | EC50 ng/mL |
|-----------------|----------------|------------|
| PC1014-025      | 86.7           | ~9.77      |
| PC1014-084      | 50.5           | ND         |
| PC1014-091      | 83.3           | ~9.86      |
| PC1014-098      | 87.2           | 1.65       |
| PC1014-129      | 75.9           | ~10.60     |
| PC1014-153      | 82.4           | ~10.11     |
| PC1014-169      | 34.0           | ND         |
| TH1014-pert     | 37.0           | 61.58      |
| trastuzumab     | Ndet           | Ndet       |
| isotype control | NDet           | NDet       |

#### **Example 19 - Internalization of HER2 antibodies measured with an FMAT-based fab-CypHer5E assay**

To investigate whether the enhanced killing of AU565 cells observed in the kappa-toxin-ETA' assay described in the previous Example correlated with enhanced internalization of HER2 antibodies, a fab-CypHer5E-based internalization assay was performed. CypHer5E™ is a pH sensitive dye which is non-fluorescent at basic pH (extracellular: culture medium) and fluorescent at acidic pH (intracellular: lysosomes), with an acid dissociation constant (pKa) of 7.3.

AU565 cells were seeded in 384-well tissue culture plates (Greiner bio-one), at a density of 3000 cells/well in normal cell culture medium supplemented with 240 ng/mL fab-CypHer5E (conjugation of Goat-fab-anti-Human IgG [Jackson] with CypHer5E [GE Healthcare, Eindhoven, The Netherlands] was made according to manufacturer's instructions). Next, HER2 antibodies were serially diluted in normal cell culture medium, added to the cells and left at room temperature for 9 hours. Mean fluorescent Intensities (MFI) of intracellular CypHer5E were measured using the 8200 FMAT (Applied Biosystems, Nieuwerkerk A/D IJssel, The Netherlands) and 'counts x fluorescence' was used as read-out. An isotype control antibody was used as negative control antibody. EC<sub>50</sub> values and maximal

MFI were determined by means of non-linear regression (sigmoidal dose-response with variable slope) using GraphPad Prism V4.03 software (GraphPad Software, San Diego, CA, USA).

The results are shown in Table 8, depicting the EC<sub>50</sub> and maximal MFI values for all tested HER2 antibodies in the CypHer5E internalization assay with AU565 cells. The maximal MFI values indicate how many HER2 receptors are internalized upon antibody binding. All HER2 antibodies showed higher maximal MFI values (137,904 – 38,801) compared to trastuzumab (35,000) and pertuzumab (TH1014-pert) (32,366), indicating that the tested HER2 antibodies induced enhanced receptor internalization. Notably, antibodies that did not compete with trastuzumab or TH1014-pert induced more receptor internalization compared to antibodies that did compete with trastuzumab and TH1014-pert, with the highest MFI achieved by antibodies 098 and 127. Without being limited to theory, this might be inherent to an inability to inhibit HER2 heterodimerization.

**Table 8:** Cypher-5-based internalization assay of HER2 antibodies. Data shown are MFI and EC<sub>50</sub> values of one representative experiment of two experiments with AU565 cells treated with fab-CypHer5E-labeled HER2 antibodies. Some EC<sub>50</sub> values could not be calculated (ND).

| Cypher 5    |                         |             |                                    |
|-------------|-------------------------|-------------|------------------------------------|
| Antibody    | EC <sub>50</sub> ng/ mL | Maximal MFI |                                    |
| PC1014-025  | 30.05                   | 63428       | mAbs that compete with Herceptin   |
| PC1014-091  | 32.99                   | 50711       |                                    |
| PC1014-129  | 7.15                    | 60302       |                                    |
| TH1014-pert | 530                     | 32366       |                                    |
| PC1014-169  | ND                      | 38801       | mAbs that compete with TH1014-pert |
| PC1014-084  | 30.51                   | 71059       |                                    |
| trastuzumab | 21.70                   | 35000       |                                    |
| PC1014-098  | 13.77                   | 134575      | mAbs that compete with TH1014-F5   |
| PC1014-127  | ~9.68                   | 137904      |                                    |
| PC1014-159  | ND                      | 92427       |                                    |
| TH1014-F5   | 22.65                   | 113116      |                                    |
| PC1014-132  | 11.42                   | 112270      | Non-competing mAbs                 |
| PC1014-153  | ~14.91                  | 87531       |                                    |

**Example 20: Generation of bispecific antibodies by 2-MEA-induced Fab-arm exchange**

An in vitro method for producing bispecific antibodies is described in WO 2008119353 (Genmab) and reported van der Neut-Kolfschoten et al. (Science. 2007 Sep 14;317(5844):1554-7). Herein, a bispecific antibody is formed by "Fab-arm" or "half-molecule" exchange (swapping of a heavy chain and attached light chain) between two monospecific IgG4- or IgG4-like antibodies upon incubation under mildly reducing conditions. This Fab-arm exchange reaction is the result of a disulfide-bond isomerization reaction wherein the inter heavy-chain disulfide bonds in the hinge regions of monospecific antibodies are reduced and the resulting free cysteines form a new inter heavy-chain disulfide bond with cysteine residues of another antibody molecule with a different specificity. The resulting product is a bispecific antibody having two Fab arms with different sequences.

In a novel invention the knowledge of this natural IgG4 Fab-arm exchange is adapted to generate a method to produce stable IgG1-based bispecific antibodies. The bispecific antibody product generated by this method described below will no longer participate in IgG4 Fab-arm exchange. The basis for this method is the use of complimentary CH3 domains, which promote the formation of heterodimers under specific assay conditions. To enable the production of bispecific antibodies by this method, IgG1 molecules carrying certain mutations in the CH3 domain were generated: in one of the parental IgG1 antibody T350I, K370T and F405L mutations in the other parental IgG1 antibody the K409R mutation.

To generate bispecific antibodies, these two parental antibodies, each antibody at a final concentration of 0.5 mg/mL (equimolar concentration), were incubated with 25 mM 2-mercaptoethylamine-HCl (2-MEA) in a total volume of 100 µL TE at 37°C for 90 min. The reduction reaction is stopped when the reducing agent 2-MEA is removed by using spin columns (Microcon centrifugal filters, 30k, Millipore) according to the manufacturer's protocol.

**Example 21 - HER2 x HER2 bispecific antibodies tested in an *in vitro* kappa-directed ETA' killing assay**

The example shows that HER2 x HER2 bispecific antibodies can deliver a cytotoxic agent into tumor cells after internalization in a generic *in vitro* cell-based killing assay using kappa-directed pseudomonas-exotoxin A (anti-kappa-ETA'). This assay makes use of a high affinity anti-kappa domain antibody conjugated to a truncated form of the pseudomonas-exotoxin A. Similar fusion proteins of antibody binding proteins (IgG-binding motif from

Streptococcal protein A or protein G) and diphtheria toxin or *Pseudomonas* exotoxin A have previously been (Mazor Y. et al., *J. Immunol. Methods* 2007; 321:41-59); Kuo SR. et al., 2009 *Bioconjugate Chem.* 2009; 20:1975-1982). These molecules in contrast to anti-kappa-ETA' bound the Fc part of complete antibodies. Upon internalization and endocytic sorting the anti-kappa-ETA' domain antibody undergoes proteolysis and disulfide-bond reduction, separating the catalytic from the binding domain. The catalytic domain is then transported from the Golgi to the endoplasmic reticulum via a KDEL retention motif, and subsequently translocated to the cytosol where it inhibits protein synthesis and induces apoptosis (Kreitman RJ. et. al., *BioDrugs* 2009; 23:1-13).

The anti-HER2 antibodies used in this example are **025**, **153** and **169**. In addition a fully human monoclonal IgG1, $\kappa$  antibody **005** of the following sequence was used:

**005:**

|                  |            |                                                                                                                                     |
|------------------|------------|-------------------------------------------------------------------------------------------------------------------------------------|
| SEQ ID<br>NO:164 | VH 005     | EVQLVQSGAEVKKPGESLKISCKASGYSFHFYWIGWVRQMPGKGLEWM<br>GSIYPGDS DTRYRPSFQGQVTISADKSISTAYLQWTSLKASDTAIYYCAR<br>QRGDYYYFYGMDVWGQGTTVTVSS |
| SEQ ID<br>NO:165 | VL 005     | EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWYQQKPGQVPRLLIY<br>GASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYGSSLTFGG<br>GTKVEIK                   |
| SEQ ID<br>NO:166 | VH<br>CDR1 | GYSFHFYW                                                                                                                            |
| SEQ ID<br>NO:167 | VH<br>CDR2 | YPGDSDT                                                                                                                             |
| SEQ ID<br>NO:168 | VH<br>CDR3 | ARQRGDYYYFYGMDV                                                                                                                     |
| SEQ ID<br>NO:169 | VL<br>CDR1 | QSVSSSY                                                                                                                             |
|                  | VL<br>CDR2 | GAS                                                                                                                                 |
| SEQ ID<br>NO:170 | VL<br>CDR3 | QQYGSSLT                                                                                                                            |

The following antibodies were used as starting materials:

IgG1-005-ITL = **005** IgG1, $\kappa$  having Ile at position 350, Thr at position 370, and Leu at position 405

IgG1-005-K409R = **005** IgG1, $\kappa$  having an Arg at position 409

IgG1-025-ITL = **025** IgG1, $\kappa$  having Ile at position 350, Thr at position 370, and Leu at position 405

IgG1-153-ITL = **153** IgG1, $\kappa$  having contains Ile at position 350, Thr at position 370, and Leu at position 405

IgG1-153-K409R = **153** IgG1, $\kappa$  having an Arg at position 409

IgG1-169-K409R = **169** IgG1, $\kappa$  having an Arg at position 409

The following bispecific antibodies were generated in analogy with the below procedure:

IgG1-005-ITL x IgG1-169-K409R

IgG1-025-ITL x IgG1-005-K409R

IgG1-025-ITL x IgG1-153-K409R

IgG1-025-ITL x IgG1-169-K409R

IgG1-153-ITL x IgG1-005-K409R

IgG1-153-ITL x IgG1-169-K409R

The bispecific antibodies were produced according to the procedure described in example 20.

The antibody mixtures, containing each antibody at a final concentration of 0.5 mg/mL, was incubated with 25 mM 2-mercaptoethylamine HCl (2-MEA) in a total volume of 100  $\mu$ L TE at 37°C for 90 min. To stop the reduction reaction, the reducing agent 2-MEA was removed by desalting the samples using spin columns (Microcon™ centrifugal filters, 30k, Millipore) according to the manufacturer's recommendations.

The HER2 x HER2 bispecific antibodies were pre-incubated with the anti-kappa-ETA' before incubation with A431 cells. A431 cells express ~15,000 HER2 antibodies per cell (determined via Qifi analysis) and are not sensitive to treatment with 'naked' HER2-antibodies.

First, the optimal concentration of anti-kappa-ETA' was determined for each cell line, i.e. the maximally tolerated dose that does not lead to induction of non-specific cell death. A431 cells (2500 cells/well) were seeded in normal cell culture medium in a 96-wells tissue culture plate (Greiner bio-one) and allowed to adhere for at least 4 hours. These cells were incubated with an anti-kappa-ETA' dilution series, 100, 10, 1, 0.1, 0.01, 0.001 and 0  $\mu$ g/mL in normal cell culture medium. After 3 days, the amount of viable cells was quantified with Alamarblue (BioSource International, San Francisco, US) according to the manufacturer's instruction. Fluorescence was monitored using the EnVision 2101 Multilabel reader (PerkinElmer, Turku, Finland) with standard Alamarblue settings. The highest concentration anti-kappa-ETA' that did not kill the cells by itself (1  $\mu$ g/mL for A431 cells) was used for following experiments.

Next, the effect of HER2 x HER2 bispecific antibodies and HER2 monospecific antibodies pre-incubated with anti-kappa-ETA' was tested for their ability to induce cell kill. A431 cells were seeded as described above. A dilution series of the HER2 specific antibodies

(monospecific and bispecific antibodies) was made and pre-incubated for 30 min with the predetermined concentration of anti-kappa-ETA' before adding them to the cells. After 3 days incubation at 37°C, the amount of viable cells was quantified as described above. The Alamarblue signal of cells treated with anti-kappa-ETA' pre-incubated with the antibodies was plotted compared to cells treated without antibody treatment. EC<sub>50</sub> values and maximal cell death were calculated using GraphPad Prism 5 software. Staurosporin (23.4 µg/mL) was used as positive control for cell killing. An isotype control antibody (IgG1/kappa; IgG1-3G8-QITL) was used as negative control.

Figure 9 and table 9 shows that all anti-kappa-ETA' pre-incubated HER2 bispecific antibodies were able to kill A431 cells in a dose-dependent manner. These results demonstrate that most HER2 bispecific antibodies tested were more effective than the monospecific antibody present in the combination in this anti-kappa-ETA' assay. In addition, the efficacy of bispecific antibody 005X169, 025X169 and 153X169 showed that the efficacy of a monospecific antibody which lacks activity in this *in vitro* kappa-directed ETA' killing, HER2 specific antibody (169), can be increased through bispecific combination with another HER2 specific antibody.

**Table 9:** EC<sub>50</sub> values and maximal percentage cell kill of AU565 cells treated with anti-kappa-ETA'-conjugated HER2 x HER2 bispecific antibodies. "Ndet" means not detected.

| antibody                      | percentage kill | EC50 [ng/mL] |
|-------------------------------|-----------------|--------------|
| Herceptin                     | 2.79            | Ndet         |
| IgG1-005-ITL                  | 79.34           | 2.57         |
| IgG1-005-K409R                | 79.83           | 2.87         |
| IgG1-025-ITL                  | 69.81           | 3.76         |
| IgG1-153-ITL                  | 70.66           | 12.45        |
| IgG1-153-K409R                | 72.84           | 15.47        |
| IgG1-169-K409R                | 16.45           | 3.45         |
| IgG1-005-ITL x IgG1-169-K409R | 59.94           | 4.28         |
| IgG1-025-ITL x IgG1-005-K409R | 63.45           | 4.27         |
| IgG1-025-ITL x IgG1-153-K409R | 80.82           | 7.66         |
| IgG1-025-ITL x IgG1-169-K409R | 45.88           | 7.97         |
| IgG1-153-ITL x IgG1-005-K409R | 80.05           | 4.51         |
| IgG1-153-ITL x IgG1-169-K409R | 84.68           | 29.14        |

**Example 22 - HER2 receptor downmodulation by incubation with bispecific antibodies targeting different HER2 epitopes**

HER2 x HER2 bispecific antibodies may bind two different epitopes on two spatially different HER2 receptors. This may allow other HER2 x HER2 bispecific antibodies to bind to the remaining epitopes on these receptors. This could result in multivalent receptor cross-

linking (compared to dimerization induced by monovalent antibodies) and consequently enhance receptor downmodulation. To investigate whether HER2 x HER2 bispecific antibodies induce enhanced downmodulation of HER2, AU565 cells were incubated with antibodies and bispecific antibodies for three days. Total levels of HER2 and levels of antibody bound HER2 were determined.

AU565 cells were seeded in a 24-well tissue culture plate (100.000 cells / well) in normal cell culture medium and cultured for three days at 37°C in the presence of 10 µg/mL HER2 antibody with either the ITL or the K409R mutation or HER2 x HER2 bispecific antibodies. As a control, the combination of two monospecific HER2 antibodies, with unmodified IgG1 backbones, was also tested (1:1), at a final concentration of 10 µg/mL. After washing with PBS, cells were lysed by incubating them for 30 min at room temperature with 25 µL Surefire™ Lysis buffer (Perkin Elmer, Turku, Finland). Total protein levels were quantified using bicinchoninic acid (BCA) protein assay reagent (Pierce) following manufacturer's protocol. HER2 protein levels in the lysates were analyzed using a HER2-specific sandwich ELISA. Rabbit-anti-human HER2 intracellular domain antibody (Cell Signaling) was used to capture HER2 and biotinylated goat-anti-human HER2 polyclonal antibody R&D systems, Minneapolis, USA), followed by streptavidin-poly-HRP, were used to detect bound HER2. The reaction was visualized using 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (one ABTS tablet diluted in 50 mL ABTS buffer [Roche Diagnostics, Almere, The Netherlands]) and stopped with oxalic acid (Sigma-Aldrich, Zwijndrecht, The Netherlands). Fluorescence at 405 nm was measured on a microtiter plate reader (Biotek Instruments, Winooski, USA) and the amount of HER2 was expressed as a percentage relative to untreated cells.

The results are shown in Figure 10 and Table 10 which demonstrates that all the tested HER2 x HER2 bispecific antibodies induced  $\geq 40\%$  HER2 downmodulation. Interestingly, all HER2 x HER2 bispecific antibodies demonstrated increased HER2 downmodulation compared to both of their monospecific counterparts.

**Table 10:** HER2 x HER2 bispecific induced downmodulation of HER2 depicted as percentage HER2 compared to untreated cells

| antibody                      | % HER2 compared to untreated cells |
|-------------------------------|------------------------------------|
| Herceptin                     | 71                                 |
| IgG1-005-ITL                  | 54                                 |
| IgG1-005-K409R                | 50                                 |
| IgG1-025-ITL                  | 64                                 |
| IgG1-153-ITL                  | 43                                 |
| IgG1-153-K409R                | 40                                 |
| IgG1-169-K409R                | 64                                 |
| IgG1-005-ITL x IgG1-169-K409R | 29                                 |
| IgG1-025-ITL x IgG1-005-K409R | 38                                 |
| IgG1-025-ITL x IgG1-153-K409R | 29                                 |
| IgG1-025-ITL x IgG1-169-K409R | 34                                 |
| IgG1-153-ITL x IgG1-005-K409R | 23                                 |
| IgG1-153-ITL x IgG1-169-K409R | 28                                 |
| IgG1-005 + IgG1-169           | 28                                 |
| IgG1-025 + IgG1-005           | 28                                 |
| IgG1-025 + IgG1-153           | 23                                 |
| IgG1-025 + IgG1-169           | 25                                 |
| IgG1-153 + IgG1-005           | 23                                 |
| IgG1-153 + IgG1-169           | 23                                 |
| isotype control               | 108                                |

**Example 23 - Colocalization of HER2 x HER2 bispecific antibodies with lysosomal marker LAMP1 analyzed by confocal microscopy**

The HER2 downmodulation assay as described in Example 22 indicated that HER2 x HER2 bispecific antibodies were able to increase lysosomal degradation of HER2. To confirm these findings, confocal microscopy technology was applied. AU565 cells were grown on glass coverslips (thickness 1.5 micron, Thermo Fisher Scientific, Braunschweig, Germany) in standard tissue culture medium at 37°C for 3 days. Cells were pre-incubated for 1 hour with 50 µg/mL leupeptin (Sigma) to block lysosomal activity after which 10 µg/mL HER2 monospecific antibodies or HER2 x HER2 bispecific antibodies were added. Also the combination of two monospecific IgG1 antibodies (1:1) was tested at a final concentration of 10 µg/mL. The cells were incubated for an additional 3 or 18 hours at 37°C. Hereafter the cells were washed with PBS and incubated for 30 min. at room temperature with 4% formaldehyde (Klinipath). Slides were washed with blocking buffer (PBS supplemented with 0.1% saponin [Roche] and 2% BSA [Roche]) and incubated for 20 min with blocking buffer containing 20 mM NH<sub>4</sub>Cl to quench formaldehyde. Slides were washed again with blocking buffer and incubated for 45 min at room temperature with mouse-anti-human CD107a (LAMP1) (BD Pharmingen) to stain/identify lysosomes. Following washing with blocking buffer, the slides were incubated 30 min at room temperature with a cocktail of secondary antibodies; goat-anti-mouse IgG-Cy5 (Jackson) and goat-anti-human IgG-FITC (Jackson). Slides were washed again with blocking buffer and mounted overnight on microscope slides using 20 µL mounting medium (6 gram Glycerol [Sigma] and 2.4 gram Mowiol 4-88

[Omnilabo] was dissolved in 6 mL distilled water to which 12 mL 0.2M Tris [Sigma] pH8.5 was added followed by incubation for 10 min at 50-60°C. Mounting medium was aliquoted and stored at -20°C.). Slides were imaged with a Leica SPE-II confocal microscope (Leica Microsystems) equipped with a 63x 1.32-0.6 oil immersion objective lens and LAS-AF software. To allow for quantification of overlapping pixel intensities, saturation of pixels should be avoided. Therefore the FITC laser intensity was decreased to 10%, smart gain was set at 830 V and smart offset was set at -9.48 %. By using these settings, the bispecific antibodies were clearly visualized without pixel saturation, but the monospecific antibodies were sometimes difficult to detect. To compare lysosomal colocalization between monospecific and bispecific antibodies, these settings were kept the same for all analyzed confocal slides.

12-bit grayscale TIFF images were analyzed for colocalisation using MetaMorph® software (version Meta Series 6.1, Molecular Devices Inc, Sunnyvale California, USA). FITC and Cy5 images were imported as stacks and background was subtracted. Identical thresholds settings were used (manually set) for all FITC images and all Cy5 images. Colocalisation was depicted as the pixel intensity of FITC in the region of overlap (ROI), where the ROI is composed of all Cy5 positive regions. To compare different slides stained with several HER2 antibodies, HER2 x HER2 bispecific antibodies or the combination of two different monospecific antibodies the images were normalized using the pixel intensity of Cy5. Goat-anti-mouse IgG-Cy5 was used to stain the lysosomal marker LAMP1 (CD107a). The pixel intensity of LAMP1 should not differ between various HER2 antibodies or the HER2 x HER2 bispecific antibodies tested (one cell had a pixel intensity of Cy5 of roughly 200.000).

Normalized values for colocalization of FITC and Cy5 =

$$[(TPI-FITC \times \text{percentage FITC-Cy5 colocalization})/100] \times [200.000/TPI-Cy5]$$

In this formula, TPI stands for Total Pixel Intensity.

Figure 11 and Table 11 present percentage of viable cells, as measured by the FITC pixel intensity overlapping with Cy5 for various monospecific HER2 antibodies and HER2 x HER2 bispecific antibodies. For each antibody or bispecific molecule depicted, three different images were analyzed from one slide containing ~ 1, 3 or >5 cells. Significant variation was observed between the different images within each slide. However, it was evident that all HER2 x HER2 bispecific antibodies demonstrate increased colocalisation with the lysosomal marker LAMP1, when compared with their monospecific counterparts. These results indicate

that once internalized, HER2 x HER2 bispecific antibodies are efficiently sorted towards lysosomal compartments, making them suitable for a bispecific antibody drug conjugate approach.

**Table 11:** Mean FITC pixel intensities overlapping with Cy5 depicted as arbitrary units

| antibody                      | FITC pixel intensity in lysosomes [arbitrary units] |
|-------------------------------|-----------------------------------------------------|
| Herceptin                     | 0.218                                               |
| IgG1-005-ITL                  | 0.070                                               |
| IgG1-025-ITL                  | 0.268                                               |
| IgG1-153-ITL                  | 0.102                                               |
| IgG1-169-K409R                | 0.220                                               |
| IgG1-005-ITL x IgG1-169-K409R | 0.531                                               |
| IgG1-025-ITL x IgG1-005-K409R | 0.347                                               |
| IgG1-025-ITL x IgG1-153-K409R | 0.582                                               |
| IgG1-025-ITL x IgG1-169-K409R | 0.439                                               |
| IgG1-153-ITL x IgG1-005-K409R | 0.494                                               |
| IgG1-153-ITL x IgG1-169-K409R | 0.604                                               |
| IgG1-025 + IgG1-169           | 0.576                                               |
| IgG1-153 + IgG1-005           | 0.636                                               |
| IgG1-153 + IgG1-169           | 0.626                                               |

**Example 24 - Inhibition of proliferation of AU565 cells upon incubation with HER2 monospecific or HER2 X HER2 bispecific antibodies**

The HER2 x HER2 bispecific antibodies were tested for their ability to inhibit proliferation of AU565 cells *in vitro*. Due to the high HER2 expression levels on AU565 cells (~1.000.000 copies per cell as determined with Qifi-kit™), HER2 is constitutively active in these cells and thus not dependent on ligand-induced heterodimerization. In a 96-wells tissue culture plate (Greiner bio-one, Frickenhausen, Germany), 9.000 AU565 cells were seeded per well in the presence of 10 µg/mL HER2 antibody or HER2 x HER2 bispecific antibodies in serum-free cell culture medium. As a control, cells were seeded in serum-free medium without antibody or bispecific antibodies. After three days, the amount of viable cells was quantified with Alamarblue (BioSource International, San Francisco, US) according to the manufacturer’s instructions. Fluorescence was monitored using the EnVision 2101 Multilabel reader (PerkinElmer, Turku, Finland) with standard Alamarblue settings. The Alamarblue signal of antibody-treated cells was plotted as a percentage relative to untreated cells.

Figure 12 and table 12 depicts the fluorescent intensity of Alamarblue of AU565 cells after incubation with HER2 antibodies and HER2XHER2 bispecific antibodies. Herceptin® (trastuzumab) was included as positive control and demonstrated inhibition of proliferation as described by Juntilla TT. et al., *Cancer Cell* 2009; 15: 429-440. All HER2 x HER2 bispecific antibodies were able to inhibit proliferation of AU565 cells. Bispecific antibodies:

IgG1-005-ITL x IgG1-169-K409R and IgG1-025-ITL x IgG1-005-K409R were more effective compared to their monospecific antibody counterparts in this assay.

**Table 12:** Percentage viable AU565 cells after treatment with HER2 x HER2 bispecific antibodies.

| antibody                      | percentage viable cells |
|-------------------------------|-------------------------|
| Herceptin                     | 62                      |
| IgG1-005-ITL                  | 91                      |
| IgG1-005-K409R                | 96                      |
| IgG1-025-ITL                  | 79                      |
| IgG1-153-ITL                  | 98                      |
| IgG1-153-K409R                | 97                      |
| IgG1-169-K409R                | 63                      |
| IgG1-005-ITL x IgG1-169-K409R | 49                      |
| IgG1-025-ITL x IgG1-005-K409R | 61                      |
| IgG1-025-ITL x IgG1-153-K409R | 74                      |
| IgG1-025-ITL x IgG1-169-K409R | 76                      |
| IgG1-153-ITL x IgG1-005-K409R | 71                      |
| IgG1-153-ITL x IgG1-169-K409R | 77                      |
| isotype control               | 95                      |

**Example 25 - Her2 x CD3 bispecific antibodies tested in an *in vitro* cytotoxicity assay**

CD3 is a co-receptor in the T cell receptor complex expressed on mature T cells. Combination of a CD3 specific antibody Fab-arm with a tumor antigen specific antibody Fab-arm in a bispecific antibody would result in the specific targeting of T cells to tumor cells, leading to T cell mediated tumor cell lysis. Likewise, CD3 positive T cells could be targeted to other derailed cells in the body, to infected cells or directly to pathogens.

HER2 x CD3 bispecific antibodies were generated. Heavy and light chain variable region sequences for the HER2 specific Fab-arm were as indicated for antibody 153 and 169 in Example 21. The following heavy and light chain variable region sequences for the CD3 specific Fab-arm were used:

**YTH12.5** (Sequence as described by Routledge et al., Eur J Immunol. 1991, 21(11):2717-25.)

|                |            |                                                                                                                            |
|----------------|------------|----------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO: 171 | VH YTH12.5 | EVQLLESGGGLVQPGGSLRLSCAASGFTFSSFPMAWVRQAPGKGLEWVSTI STSGGRYYRDSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKFRQYS GGF DYWGQGT LTVSS |
| SEQ ID NO: 172 | VL YTH12.5 | DIQLTQPN SVSTSLGSTVKLSCTLSSGNIENNYVHWYQLYEGRSPTTMIYDD DKRPDGV PDRFSGSIDRSSNSAFLTIHNVAIEDEAIYFCHSYVSSFN VF GGGT KLTVL       |

**huCLB-T3/4** (Sequence as described by Parren et al., Res Immunol. 1991, 142(9):749-63. Minor amino acid substitutions were introduced to make the sequence resemble the closest human germline.)

|                |                  |                                                                                                                         |
|----------------|------------------|-------------------------------------------------------------------------------------------------------------------------|
| SEQ ID NO: 173 | VH<br>huCLB-T3/4 | EVQLVESGGGLVKPGGSLRLSCAASGFTFSSYGMFWVRQAPGKGLEWVATISRYSRYIYYPDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARRPLYGSSPDYWGQGTLVTVSS |
| SEQ ID NO: 174 | VL<br>huCLB-T3/4 | EIVLTQSPATLSLSPGERATLSCSASSSVTYVHWYQQKPGQAPRLLIYDTSKLASGIPARFSGSGSGTDFTLTISSLEPEDFAVYYCFQSGSGYPLTFGSGTKLEMR             |

All antibodies were expressed as IgG1, $\kappa$  being modified in their Fc regions as follows: IgG1-HER2-153-K409R and IgG1- HER2-153-N297Q-K409R, IgG1-HER2-169-K409R, IgG1-hu-CLB-T3/4-F405L and IgG1-hu-CLB-T3/4-N297Q-F405L, IgG1-YTH12.5-F405L and IgG1-YTH12.5-N297Q-F405L.

Bispecific antibodies from these HER2 and CD3 specific antibodies were generated as described in Example 20 and tested in an *in vitro* cytotoxicity assay using AU565 cells. AU565 cells were cultured to near confluency. Cells were washed twice with PBS, and trypsinized for 5 minutes at 37°C. 12 mL culture medium was added to inactivate trypsin and cells were spun down for 5 min, 800 rpm. Cells were resuspended in 10 mL culture medium and a single cell suspension was made by passing the cells through a cellstrainer. 100  $\mu$ L of a 5x10<sup>5</sup> cells/mL suspension was added to each well of a 96-well culture plate, and cells were incubated at least 3 hrs at 37°C, 5% CO<sub>2</sub> to allow adherence to the plate. Peripheral blood mononuclear cells (PBMC) were isolated from blood from healthy volunteers using Leucosep™ 30 mL tubes, according to the manufacturer's protocol (Greiner Bio-one). T cells were isolated from PBMC preparations by negative selection using the Untouched Human T-cells Dynabead™ kit (Dyna). Isolated cells were resuspended in culture medium to a final concentration of 7x10<sup>6</sup> cells/mL.

Culture medium was removed from the adhered AU565 cells, and replaced with 50  $\mu$ l/well 2x concentrated antibody-dilution and 50  $\mu$ l/well 7x10<sup>6</sup> T cells/mL (ratio effector:target = 7:1). Plates were incubated for 3 days at 37°C, 5% CO<sub>2</sub>. Supernatants were removed and plates were washed twice with PBS. To each well 150  $\mu$ L culture medium and 15  $\mu$ L Alamar blue was added. Plates were incubated for 4 hours at 37°C, 5% CO<sub>2</sub>, and absorbance was measured (Envision, Perkin Elmer).

Figure 13 and table 13 shows that whereas control antibodies (Her2 monospecific IgG1-Herceptin®, CD3 monospecific IgG1-YTH12.5 and monospecific IgG1-huCLB-T3/4, irrelevant antigen monospecific IgG1-b12, and CD3 x b12 bispecific antibodies) did not

induce T cell mediated cytotoxicity, bispecific (Duo) Her2 x CD3 antibodies huCLB/Her2-153, huCLB/Her2-169, YTH12.5/Her2-153 and YTH12.5/Her2-169 induced dose dependent T cell mediated cytotoxicity of AU565 cells. Bispecific antibodies containing Her2-169 were more potent than those containing Her2-153.

Mutants of IgG1-hu-CLB-T3/4, IgG1-YTH12.5 and Her2-153 were made containing a N297Q mutation to remove a glycosylation site; glycosylation at this site is critical for IgG-Fcγ receptor interactions (Bolt S et al., *Eur J Immunol* 1993, 23:403-411). Figure 13 shows that N297Q mutation and therefore absence of Fc glycosylation of Her2 x CD3 bispecific antibodies YTH12.5/Her2-153 and huCLB/Her2-153 did not impact the potential to induce dose dependent T cell mediated cytotoxicity of AU565 cells.

**Table 13:** EC<sub>50</sub> values of cell kill induced by HER2 x CD3 bispecific antibodies. "Ndet" means not detected.

| antibody            | EC50 [ng/mL] |
|---------------------|--------------|
| Herceptin           | Ndet         |
| Duo huCLB-Q/153-Q   | 10.55        |
| Duo huCLB-Q/B12-Q   | Ndet         |
| huCLB-Q             | Ndet         |
| B12-Q               | Ndet         |
| Duo YTH12.5-Q/153-Q | 10.73        |
| Duo YTH12.5-Q/B12-Q | Ndet         |
| YTH12.5-Q           | Ndet         |
| B12-Q               | Ndet         |

#### Example 26 - HER2 downmodulation

To investigate if enhanced HER2 internalization induced by Group 3 antibodies 098 and 153 also results in enhanced receptor downmodulation, AU565 cells were incubated with HER2 antibodies for 3 days, and analyzed for presence of HER2. AU565 cells were seeded in a 24-wells tissue culture plate (100.000 cells/well) in normal cell culture medium and cultured for 3 days at 37°C in the presence of 10 µg/mL HER2 antibody. After washing with PBS, cells were lysed by incubating 30 min at room temperature with 25 µL Surefire Lysis buffer (Perkin Elmer, Turku, Finland). Total protein levels were quantified using bicinchoninic acid (BCA) protein assay reagent (Pierce) according to the manufacturer's protocol. HER2 protein levels in the lysates were analyzed using a HER2-specific sandwich ELISA. Rabbit-anti-human HER2 intracellular domain antibody (Cell Signaling) was used to capture HER2 and biotinylated goat-anti-human HER2 polyclonal antibody (R&D), followed by streptavidin-poly-HRP, were used to detect bound HER2. The reaction was visualized using 2,2'-azino-bis 3-ethylbenzothiazoline-6-sulfonic acid (ABTS: dilute one ABTS tablet in 50 mL ABTS buffer [Roche Diagnostics, Almere, The Netherlands]) and stopped with oxalic acid (Sigma-Aldrich,

Zwijndrecht, The Netherlands). Fluorescence at 405 nm was measured on a microtiter plate reader (Biotek Instruments, Winooski, USA) and the amount of HER2 was expressed as a percentage relative to untreated cells.

The results shown in Figure 14 and Table 14 demonstrate that both Group 3 antibodies (098 and 153) induced more than 50% HER2 downmodulation. In contrast, antibodies 025, 169 and Herceptin barely induced downmodulation (approximately 20% of untreated cells). This was in line with enhanced internalization observed by antibodies 098 and 153.

**Table 14:** Antibody induced downmodulation of HER2 depicted as percentage HER2 compared to untreated cells

| antibody        | % HER2 compared to untreated cells |
|-----------------|------------------------------------|
| Herceptin       | 80                                 |
| IgG1-1014-169   | 82                                 |
| IgG1-1014-025   | 85                                 |
| IgG1-1014-098   | 44                                 |
| IgG1-1014-153   | 50                                 |
| isotype control | 108                                |

**Example 27 - Colocalization of HER2 antibodies with lysosomal marker LAMP1 analyzed by confocal microscopy.**

The HER2 downmodulation assay as described in example 26 and the CypHer-5E based internalization assay as described in example 19 indicated that HER2 antibodies from group 3 were more efficiently internalized and targeted towards lysosomes compared to antibodies from Groups 1 and 2. However, in these experiments the confocal imaging was done with settings that allowed discriminating between monospecific and bispecific antibodies but not between different monospecific antibodies, in fact, with these settings monospecific antibodies could hardly be detected. To be able to compare between the different monospecific antibodies, the confocal slides were measured again with increased gain settings, to enhance fluorescence intensity. All other steps of the procedure were the same as described in example 23.

The results are depicted in Figure 15 and Table 15, and show that the FITC pixel intensity overlapping with Cy5 for various monospecific HER2 antibodies. From each slide three different images were analyzed containing ~ 1, 3 or >5 cells. Significant variation was observed between the different images within each slide. Still, it was evident that antibodies 098 and 153 were more efficiently targeted towards lysosomal compartments, compared to 025, pertuzumab, 169 and Herceptin. This correlated well with the enhanced internalization and receptor degradation induced by these antibodies.

**Table 15:** Mean FITC pixel intensities overlapping with Cy5 depicted as arbitrary units

| antibody    | FITC pixel intensity in lysosomes [arbitrary units] |
|-------------|-----------------------------------------------------|
| TH1014-098  | 0.522                                               |
| TH1014-153  | 0.409                                               |
| TH1014-025  | 0.248                                               |
| TH1014-pert | 0.214                                               |
| TH1014-169  | 0.255                                               |
| Herceptin   | 0.236                                               |

**Example 28 - HER2 extracellular domain shuffle human-to-chicken**

To further define the HER2 binding regions recognized by antibodies from the four different cross-competition groups, a HER2 extracellular domain shuffle experiment was performed. To this end, a small gene-synthesis library with five constructs was generated, swapping the sequences of domain I, II, III or IV of the extracellular domain of human HER2 to the corresponding sequence of chicken HER2 (Gallus gallus isoform B NCBI: NP\_001038126.1): 1) fully human HER2 (Uniprot P04626) hereafter named hu-HER2, 2) hu-HER2 with chicken domain I (replacing amino acids (aa) 1-203 of the human Her2 with the corresponding chicken Her2 region) hereafter named hu-HER2-ch(I), 3) hu-HER2 with chicken domain II (replacing amino acids (aa) 204-330 of the human Her2 with the corresponding chicken Her2 region) hereafter named hu-HER2-ch(II), 4) hu-HER2 with chicken domain III (replacing aa 331-507 of the human Her2 with the corresponding chicken Her2 region) hereafter named hu-HER2-ch(III) and 5) hu-HER2 with chicken domain IV (replacing aa 508-651 of the human Her2 with the corresponding chicken Her2 region) hereafter named hu-HER2-ch(IV). The human and chicken HER2 orthologs show 67% homology in their extracellular domain with 62% homology in domain I, 72% homology in domain II, 63% homology in domain III and 68% homology in domain IV. The constructs were transiently transfected in the Freestyle™ CHO-S (Invitrogen) cell line using Freestyle MAX transfection reagent (Invitrogen) according to the instructions of the manufacturer, and transfected cells were cultured for 20 hours. HER2 antibody binding to the transfected cells was analyzed by means of flow cytometry: The transfected CHO-S cells were harvested, washed with FACS buffer and incubated with 10 µg/mL HER2 antibody (30 minutes on ice). Binding of HER2 antibodies was detected using a Phycoerythrin (PE)-conjugated goat-anti-human IgG antibody (Jackson). To check if expression between different batches was the same, cells were fixed and permeabilized using Cytofix/Cytoperm™ solution (BD) according manufacturer's instruction and stained with a rabbit-anti-human intracellular HER2 antibody (DAKO) in combination with a secondary PE-conjugated goat-anti-rabbit antibody (Jackson). An isotype control antibody was used as negative control. Fluorescence was measured on a FACSCanto-II™ (BD) and binding curves were made by means of non-linear regression

(sigmoidal dose-response with variable slope) using GraphPad Prism V4.03 software (GraphPad Software, San Diego, CA, USA). Loss of binding was used as read out to identify which HER2 domains were recognized by the different antibodies.

Exemplary binding curves for antibody 153 are shown in figure 16. All binding results are shown in Table 16. Group 1 HER2 antibodies 050, 084, 169 and Herceptin showed loss of binding to Hu-HER2-ch(IV), but not to the proteins with one of the remaining domains shuffled, demonstrating that the epitopes of Group 1 mAbs reside in HER2 domain IV. Group 2 antibodies 025, 091, 129 and pertuzumab showed only loss of binding to Hu-HER2-ch(II), indicating that the epitope resides in HER2 domain II. Antibodies 098 and 153 were both defined to Group 3 in the cross-competition assay but showed some variation in the shuffle experiment. Antibody 098 clearly showed loss of binding to Hu-HER2-ch(I) and a minor decrease in binding to Hu-HER2-ch(II), while 153 showed only loss of binding to Hu-HER2-ch(II). These data suggest that Group 3 mAbs 098 and 153 can also bind, at least partially, to the HER2 domain II, with epitopes that possibly extend into HER2 domain I, as is the case for 098.

**Table 16:** Summary of HER2 antibody binding to different HER2ECD receptor constructs. FL; hu-HER2, I; hu-HER2-ch(I), II; hu-HER2-ch(II), III; hu-HER2-ch(III), IV; hu-HER2-ch(IV). +++ indicates normal binding, ++ indicates reduced EC<sub>50</sub> but the similar maximal binding compared to binding observed to hu-HER2, + indicates reduced EC<sub>50</sub> and reduced maximal binding detected compared to binding observed to hu-HER2, - indicates no binding.

| Antibody   | Group | HER2-domain shuffled |     |     |     |     |
|------------|-------|----------------------|-----|-----|-----|-----|
|            |       | FL                   | I   | II  | III | IV  |
| Herceptin  | 1     | +++                  | +++ | +++ | +++ | -   |
| 050        | 1     | +++                  | +++ | +++ | +++ | -   |
| 084        | 1     | +++                  | +++ | +++ | +++ | -   |
| 169        | 1     | +++                  | +++ | +++ | +++ | +   |
| Pertuzumab | 2     | +++                  | +++ | +   | +++ | +++ |
| 025        | 2     | +++                  | +++ | -   | +++ | +++ |
| 091        | 2     | +++                  | +++ | -   | +++ | +++ |
| 129        | 2     | +++                  | +++ | -   | +++ | +++ |
| 153        | 3     | +++                  | +++ | -   | +++ | +++ |
| 098        | 3     | +++                  | -   | ++  | +++ | +++ |

**Example 29 - In vivo efficacy of HER2 HuMabs 091, 084 and 169 in NCI-N87 human gastric carcinoma xenografts in SCID mice**

The in vivo effect of HER2-HuMabs 091 (cross-competition Group 2), 084 and 169 (both cross-competition Group 1) on tumor growth and survival in a NCI-N87 human gastric carcinoma xenograft model in female CB.17 severe combined immunodeficiency (SCID) mice was determined.  $10 \times 10^6$  NCI-N87 tumor cells in 50% matrigel were injected s.c. in female SCID mice, 10 mice per group. Eight days after tumor inoculation, intravenous treatment with HER2-HuMabs 091, 084, and 169 or control antibody HuMab-HepC was started. In figure 17 (A) this is indicated as day 1, day of treatment initiation. The first dose was at 40 mg/kg, followed by 10 mg/kg on days 4, 8, 11, 15, 18, 22, and 25 after treatment initiation.

Tumor volume was determined at least 2 times per week. Volumes ( $\text{mm}^3$ ) were calculated from caliper (PLEXX) measurements as  $(\text{width}^2 \times \text{length})/2$ .

The results are depicted in Figure 17A and 17B which shows that the mice administered with HuMab 084, 169 and 091 demonstrated slower tumor growth (A) and better survival (B) than the mice that received negative control antibody HuMab-HepC. All treatments were well-tolerated.

#### **Example 30 - Therapeutic treatment of BT-474 breast tumor xenografts in Balb/ C nude mice**

The effect of therapeutic treatment of five different HER2 HuMabs on human subcutaneous BT-474 breast tumor xenografts in Balb/C nude mice was determined. BT-474 tumor cells were injected 24 to 72 hours after a whole body irradiation with a  $\gamma$ -source (1.8 Gy, Co60, BioMep, France).  $2 \times 10^7$  BT-474 cells in 200  $\mu\text{l}$  of RPMI 1640 containing matrigel (50:50, v:v; BD Biosciences) were injected subcutaneously into the right flank of female Balb/C nude mice. Body weight and tumor volume of the mice was recorded twice a week. Tumor volumes ( $\text{mm}^3$ ) were calculated from caliper (PLEXX) measurements as:  $(\text{width}^2 \times \text{length})/2$ .

Treatment with HER2 HuMabs was started when the tumors reached a mean volume of 100-200  $\text{mm}^3$ . Tumor bearing mice were randomized into groups of 8 mice. One group received twice weekly intravenous (i.v.) injections of the control mAb HuMab-HepC. Four other groups received twice weekly i.v. injections of HER2 HuMab 025, 129, 153 and 091, with a first dose of 20 mg/kg and following 9 doses of 5 mg/kg.

The results are depicted in Figure 18A and 18B and show that BT-474 tumor growth was partially inhibited with HuMab 129 and HuMab 153 treatment (about 30 and 50% of inhibition compared to HuMab-HepC control treatment). HuMab-025 and HuMab-091 strongly inhibited the BT-474 tumor growth and the time to reach a tumor volume of 800

mm<sup>3</sup> was significantly delayed by these antibodies. Survival was also improved in the HER2 HuMAb receiving mice.

## CLAIMS:

1. A monoclonal antibody which binds human epidermal growth factor receptor 2 (HER2) and which comprises a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOS:134, 135, and 136, respectively, and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:27, AAS, and SEQ ID NO:28 respectively.
2. The monoclonal antibody of claim 1, wherein the VH region is derived from an IgHV4-34-1 germline.
3. The monoclonal antibody of claim 1 or 2, wherein the VL region is derived from an IgKV1D-16-01 germline.
4. The monoclonal antibody of any one of claim 1 to 3, wherein in SEQ ID NO:134, X1=D or G; and X2=Y or F.
5. The monoclonal antibody of any one of claim 1 to 4, wherein in SEQ ID NO: 135, X1=H or N; X2=S or V; and X3=T or A.
6. The monoclonal antibody of any one of claim 1 to 5, wherein in SEQ ID NO: 136, X1=Y, N or L; X2=Y or I; and X3=D, G, or A; X4=V or Y; and X5=Y or L.
7. The monoclonal antibody of any one of claim 1 to 6, wherein the VH region comprises the CDR1 sequence of SEQ ID NO: 23.
8. The monoclonal antibody of any one of claim 1 to 7, wherein the VH region comprises the CDR2 sequence of SEQ ID NO: 24.
9. The monoclonal antibody of any one of claim 1 to 8, wherein the VH region comprises the CDR2 sequence of SEQ ID NO: 25.

10. The monoclonal antibody of any one of claim 1 to 9, wherein the VH region comprises the sequence of SEQ ID NO: 22 and the VL region comprises the sequence of SEQ ID NO: 26.
11. A monoclonal antibody which binds human epidermal growth factor receptor 2 (HER2) and which comprises a VH region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NOS:152, 153, and 154, respectively, and a VL region comprising the CDR1, CDR2, and CDR3 sequences of SEQ ID NO:68, DAS, and SEQ ID NO:69 respectively.
12. The monoclonal antibody of claim 11, wherein the VH region is derived from an IgHV3-30-03-01 germline.
13. The monoclonal antibody of claim 11 or 12, wherein the VL region is derived from an IgKV1D-16-01 germline.
14. The monoclonal antibody of any one of claims 11 to 13, wherein in SEQ ID NO: 152, X=D or X; X2=Y or H; and X3=V or A.
15. The monoclonal antibody of any one of claims 11 to 14, wherein in SEQ ID NO: 153, X1=N or Y; and X2=K or E.
16. The monoclonal antibody of any one of claims 11 to 15, wherein in SEQ ID NO: 154, X1=G, D, or S; X2=I or Y; X3=T or I; X4=G or S; X5=T or S; X6=T or S; and X7=Y or V.
17. The monoclonal antibody of any one of claim 11 to 16, wherein the VH region comprises the CDR1 sequence of SEQ ID NO: 64.
18. The monoclonal antibody of any one of claim 11 to 17, wherein the VH region comprises the CDR2 sequence of SEQ ID NO: 65.
19. The monoclonal antibody of any one of claim 11 to 18, wherein the VH region comprises the CDR2 sequence of SEQ ID NO: 66.

20. The monoclonal antibody of any one of claim 11 to 19, wherein the VH region comprises the sequence of SEQ ID NO: 63 and the VL region comprises the sequence of SEQ ID NO: 67.
21. The antibody of any one of claims 1 to 20, which has an EC<sub>50</sub> value for binding to HER2-expressing cells lower than 0.80 µg/ml, when determined by flow cytometry.
22. The antibody of claim 21, which has an EC<sub>50</sub> value lower than 0.50 µg/ml.
23. The antibody of any one of claims 1 to 22, which induces ADCC, achieving a <sup>51</sup>Cr-release of at least 30%, when determined under the following conditions: (a) <sup>51</sup>Cr-labeled SK-BR-3 cells are pre-incubated with the antibody, (b) peripheral blood mononuclear cells are added to the cells of step (a) at an effector to target ration of 100:1, and (c) cell lysis is determined based on <sup>51</sup>Cr released into supernatant.
24. The antibody of claim 23, which achieves a <sup>51</sup>Cr-release of at least 40%.
25. The antibody of any one of claims 1 to 24, which inhibits ligand-independent proliferation of HER2-expressing cells when determined under the following conditions: (a) AU565 cells are seeded in the presence of HER2 antibody in serum-free cell culture medium and (b) viable cells after 3 days are quantified using a fluorescence-based viability assay.
26. The antibody of any one of claims 1 to 25, which does not promote ligand-induced proliferation of HER2-expressing cells, when determined under the following conditions: (a) MCF7 cells are seeded in complete culture medium for 4 hours, (b) the cell culture medium is replaced with starvation medium and HER2 antibody; (c) heregulin-β1 is added to the starvation medium; and (d) cell viability is determined after 4 days using a fluorescence-based viability assay.
27. The antibody of any one of claims 1 to 26, which, when conjugated directly or indirectly to a therapeutic moiety which is a truncated form of the pseudomonas-exotoxin A, kills at least 49% of HER2-expressing cells, when determined under the following conditions:

(a) a dilution series of the HER2 antibody is incubated with anti-kappa-ETA', (b) AU565 cells are incubated with the dilution series from part (a), and (c) cell viability is determined after 3 days using a fluorescence-based cell viability assay.

28. The antibody of claim 27, which kills at least 60% of the HER2-expressing cells.

29. The antibody of claim 21 or 22, wherein the HER2-expressing cells expresses less than an average of about 30000 HER2 molecules per cell.

30. The antibody of any one of claims 1 to 29, wherein a higher amount of the antibody than trastuzumab is internalized by a HER2-expressing tumor cell-line, when determined under the following conditions: (a) a dilution series of the HER2 antibody is incubated with anti-kappa-ETA', (b) AU565 cells are incubated with the dilution series from (a), and (c) cell viability is determined after 3 days using a fluorescence-based cell viability assay.

31. A bispecific antibody comprising an antigen-binding region of an antibody defined in any one of claims 1 to 30, and a second antigen-binding site having a different binding specificity.

32. The bispecific antibody of claim 31, wherein the second antigen-binding site has a binding specificity for a human effector cell, a human Fc receptor, a T cell receptor or a non-blocking epitope of HER2.

33. A bispecific antibody comprising (i) a first antibody and a (ii) second antibody, wherein the first antibody is an antibody according to any one of claims 1-30, and wherein the second antibody binds to a different epitope than the first antibody.

34. A bispecific antibody according to claim 33, wherein the second antibody is selected from the group consisting of (i) an antibody according to any one of claims 1-31, (ii) an antibody comprising a VH region comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NOs: 166, 167 and 168, respectively; and a VL region comprising CDR1, CDR2 and CDR3 sequences of SEQ ID NO: 169, GAS and SEQ ID NO: 170, and (iii) a CD3 antibody.

35. The bispecific antibody according to claim 34, wherein the second antibody comprises a VH region comprising the sequence of SEQ ID NO:164, and a VL region comprising the sequence of SEQ ID NO:165.
36. The bispecific antibody according to claim 33 or 34, wherein the CD3 antibody comprises a) a VH region comprising the sequence of SEQ ID NO:171 and a VL region comprising the sequence of SEQ ID NO:172; or b) a VH region comprising the sequence of SEQ ID NO: 173 and a VL region comprising the sequence of SEQ ID NO: 174.
37. The antibody of any one of claims 1 to 36, wherein the antibody is a full-length antibody.
38. The antibody of claim 37, which is an IgG1 antibody.
39. The antibody of claim 38, which is an IgG1, $\kappa$  antibody.
40. The antibody of any one of claims 1 to 39, wherein the antibody is conjugated to another moiety.
41. The antibody of claim 40, wherein the other moiety is a cytotoxic moiety, a radioisotope, a drug, or a cytokine.
42. The antibody of claim 41, wherein the cytotoxic moiety is selected from the group consisting of taxol; cytochalasin B; gramicidin D; ethidium bromide; emetine; mitomycin; etoposide; tenoposide; vincristine; vinblastine; colchicin; doxorubicin; daunorubicin; dihydroxy anthracin dione; a tubulin-inhibitor; mitoxantrone; mithramycin; actinomycin D; 1-dehydrotestosterone; a glucocorticoid; procaine; tetracaine; lidocaine; propranolol; puromycin; calicheamicin or an analog or derivative thereof; an antimetabolite; an alkylating agent; an antibiotic; an antimitotic agent; a toxin; ribonuclease (RNase); DNase I, Staphylococcal enterotoxin A; pokeweed antiviral protein; diphtherin toxin; and Pseudomonas endotoxin.

43. The antibody of claim 42, wherein the a tubulin-inhibitor is maytansine or an analog or derivative thereof; the antimetabolite is methotrexate, 6 mercaptopurine, 6 thioguanine, cytarabine, fludarabin, 5 fluorouracil, decarbazine, hydroxyurea, asparaginase, gemcitabine, or cladribine; the alkylating agent is mechlorethamine, thioepa, chlorambucil, melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, dacarbazine (DTIC), procarbazine, mitomycin C, cisplatin, carboplatin, duocarmycin A, duocarmycin SA, rachelmycin (CC-1065), or an analog or derivative thereof; the antibiotic is dactinomycin, bleomycin, daunorubicin, doxorubicin, idarubicin, mithramycin, mitomycin, mitoxantrone, plicamycin, anthramycin (AMC); the antimitotic agent is monomethyl auristatin E or F or an analog or derivative thereof; and the toxin is diphtheria toxin or a related molecule, ricin toxin, cholera toxin, a Shiga-like toxin, LT toxin, C3 toxin, Shiga toxin, pertussis toxin, tetanus toxin, soybean Bowman-Birk protease inhibitor, Pseudomonas exotoxin, alorin, saporin, modeccin, gelanin, abrin A chain, modeccin A chain, alpha-sarcin, Aleurites fordii proteins, dianthin proteins, Phytolacca americana protein, momordica charantia inhibitor, curcin, crotin, sapaonaria officinalis inhibitor, gelonin, mitogellin, restrictocin, phenomycin, and enomycin toxins.

44. The antibody of claim 43, wherein the related molecule is diphtheria A chain and active fragments thereof and hybrid molecules.

45. The antibody of claim 43 or 44, wherein the ricin toxin is ricin A or a deglycosylated ricin A chain toxin.

46. The antibody of any one of claims 43 to 45, wherein the Shiga-like toxin is SLT I, SLT II, or SLT IIV.

47. The antibody of any one of claims 43 to 46, wherein the Phytolacca americana protein is PAPI, PAPII, or PAP S.

48. The antibody of claim 41, which is conjugated to a cytotoxic moiety selected from the group consisting of maytansine, calicheamicin, duocarmycin, rachelmycin (CC-1065), monomethyl auristatin E, and monomethyl auristatin F, or an analog, derivative, or prodrug of any thereof.

49. The antibody of claim 41, which is conjugated to a cytokine selected from the group consisting of IL-2, IL-4, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, IL-18, IL-23, IL-24, IL-27, IL-28a, IL-28b, IL-29, KGF, IFN $\alpha$ , IFN $\beta$ , IFN $\gamma$ , GM-CSF, CD40L, Flt3 ligand, stem cell factor, anacardic acid, and TNF $\alpha$ .
50. The antibody of claim 41, which is conjugated to a radioisotope.
51. The antibody of claim 50, wherein the radioisotope is an alpha emitter.
52. A nucleic acid molecule that encodes both the VH amino acid sequence of SEQ ID NO: 22 and the VL amino acid sequence of SEQ ID NO: 26.
53. A nucleic acid molecule that encodes both the VH amino acid sequence of SEQ ID NO: 63 and the VL amino acid sequence of SEQ ID NO: 67.
54. A set of nucleic acid molecules, wherein a first nucleic acid molecule of said set encodes the VH amino acid sequence of SEQ ID NO: 22, and wherein a second nucleic acid molecule of said set encodes the VL amino acid sequence of SEQ ID NO: 26.
55. A set of nucleic acid molecules, wherein a first nucleic acid molecule of said set encodes the VH amino acid sequence of SEQ ID NO: 63, and wherein a second nucleic acid molecule of said set encodes the VL amino acid sequence of SEQ ID NO: 67.
56. An expression vector comprising a nucleotide sequence according to claim 52 or 53, wherein the vector further encodes an operably-linked constant region of a light chain, constant region of a heavy chain or both light and heavy chains of an antibody.
57. A recombinant eukaryotic or prokaryotic host cell which produces an antibody as defined in any one of claims 1 to 39.
58. A pharmaceutical composition comprising an antibody as defined in any one of claims 1 to 39 and a pharmaceutically acceptable carrier.

59. A pharmaceutical composition comprising an antibody as defined in any one of claims 40 to 51, and a pharmaceutically acceptable carrier.
60. The antibody of any one of claims 1 to 39 for use in the treatment of cancer.
61. The antibody of any one of claims 40 to 51 for use in the treatment of cancer.
62. The antibody for the use of claim 60 or 61, wherein the cancer is selected from the group consisting of breast cancer, prostate cancer, non-small cell lung cancer, bladder cancer, ovarian cancer, gastric cancer, colorectal cancer, esophageal cancer, squamous cell carcinoma of the head and neck, cervical cancer, pancreatic cancer, testis cancer, malignant melanoma and soft-tissue cancer.
63. The antibody for the use of any one of claims 60 to 62, wherein the antibody is for the treatment of cancer in combination with one or more further therapeutic agents.
64. The antibody for the use of claim 63, wherein the one or more therapeutic agents comprises a chemotherapeutic agent.
65. A use of the antibody of any one of the claims 1 to 51 for the manufacture of a medicament for treatment of cancer.
66. The use of claim 65, wherein the cancer is selected from the group consisting of breast cancer, prostate cancer, non-small cell lung cancer, bladder cancer, ovarian cancer, gastric cancer, colorectal cancer, esophageal cancer, squamous cell carcinoma of the head and neck, cervical cancer, pancreatic cancer, testis cancer, malignant melanoma and soft-tissue cancer.
67. The use of claim 65 or 66, wherein the antibody is for the treatment of cancer in combination with one or more further therapeutic agents.
68. The use of claim 67, wherein the one or more therapeutic agents comprises a chemotherapeutic agent.

69. An antibody according to any one of claims 1 to 51 for use in inhibiting growth or proliferation of one or more tumor cells expressing HER2.

70. The antibody for the use of claim 69, wherein the antibody comprises the features of any one of claims 42 to 53, and the one or more tumor cell coexpresses HER2 and EGFR, HER2 and HER3; or HER2, EGFR and HER3.

71. An antibody according to any one of claims 1 to 51 for use in treating cancer, comprising selecting a subject suffering from a cancer comprising tumor cells co-expressing HER2 and EGFR; HER2 and HER3; or HER2, EGFR and HER3, wherein the antibody is for administration to the subject.

72. The antibody for the use of claim 71, wherein the cancer is selected from the group consisting of breast cancer, colorectal cancer, endometrial/cervical cancer, lung cancer, malignant melanoma, ovarian cancer, pancreatic cancer, prostate cancer, testis cancer, a soft-tissue tumor, and bladder cancer.

73. The antibody for use of claim 72, wherein the soft tumor is synovial sarcoma.

74. A method for producing an antibody of any one of claims 1 to 51, said method comprising the steps of:

- a) culturing the recombinant host cell of claim 55 in a culture media, and
- b) purifying the antibody from the culture media.

75. A method for detecting the presence of HER2 in a sample, comprising:  
contacting the sample with an antibody of any one of claims 1 to 51 under conditions that allow for formation of a complex between the antibody and HER2; and  
analyzing whether a complex has been formed.

76. A kit for detecting the presence of HER2 in a sample comprising:  
an antibody of any one of claims 1 to 51; and  
instructions for use of the kit.

**Figure 1A**IgHV3-23-01 / IGHJ4-02 – VH alignment (Group 1)

```

IgHV1-23-01 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWRQAPGKGLEWVSAISGSGSTYYADSVKG
TH1014-050 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWRQAPGKGLEWVSAISGSGSTYYADSVKG
VH1014-049 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWRQAPGKGLEWVSAISGSGSTYYADSVKG
VH1014-051 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWRQAPGKGLEWVSAISGSGSTYYADSVKG
VH1014-055 EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWRQAPGKGLEWVSAISGSGSTYYADSVKG
Consensus EVQLLESGGGLVQPGGSLRLSCAASGFTFSSYAMSWRQAPGKGLEWVSAISGSGSTYYADSVKG

IgHV1-23-01 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAK-----YFDYWGQGTLTVTVSS
TH1014-050 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKARANWDYFDYWGQGTLTVTVSS
VH1014-049 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKARANWDYFDYWGQGTLTVTVSS
VH1014-051 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKARANWDYFDYWGQGTLTVTVSS
VH1014-055 RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKARANWDYFDYWGQGTLTVTVSS
Consensus RFTISRDNSKNTLYLQMNSLRAEDTAVYYCAKARANWDYFDYWGQGTLTVTVSS

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**Figure 1B**IgHV1-69-04 / IGHJ6-02 – VH alignment (Group 1)

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IgHV1-69-04 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKFQG
TH1014-084 QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKFQG
Consensus QVQLVQSGAEVKKPGSSVKVSCKASGGTFSSYAISWVRQAPGQGLEWMGRIIPILGIANYAQKFQG

IgHV1-69-04 RVTITADKSTSTAYMELSSLRSEDTAVYYCAR-----GMDVWGQGTTTVTVSS
TH1014-084 RVTITADKSTSTAYMELSSLRSEDTAVYYCAREKGVDYYYGELVWGQGTTTVTVSS
Consensus RVTITADKSTSTAYMELSSLRSEDTAVYYCAREKGVDYYYGELVWGQGTTTVTVSS

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**Figure 1C**IgHV1-18-01 / IGHJ4-02 – VH alignment (Group 1)

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IgHV1-18-01  QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQG
TH1014-169  QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWLISAYSGNTIYAQKLQG
VH1014-123   QVQLVQSGAEVKKPGASVKVSCKAAGYTFTNYGISWVRQAPGQGLEWMGWITTYSSNTIYAQKLQG
VH1014-161   QVQLVQSGAEVKKPGASVKVSCKASGYTFTNYGISWVRQAPGQGLEWMGWLISAYSGNTIYAQKLQG
VH1014-124   QVQLVQSGAEVKKPGASVKVSCKAAGYTFTNYGISWVRQAPGQGLEWMGWITTYNGNTIYAQRFQD
Consensus    QVQLVQSGAEVKKPGASVKVSCKASGYTFTXYGISWVRQAPGQXLEWMGWXXXXXXNTXYAQXXQG

IgHV1-18-01  RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR-----YFDYWGQGTLLVTVSS
TH1014-169  RVTMTTDTSTTTAYMELRSLRSDDTAVYYCARDRIIVRPDYFDYWGQGTLLVTVSS
VH1014-123   RVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDRVVVRPDYFDYWGQGTLLVTVSS
VH1014-161   RVTMTTDTSTTTAYMELRSLRSDDTAVYYCARDRIIVRPDYFDYWGQGTLLVTVSS
VH1014-124   RVTMTTDTSTSTAYMELRSLRSDDTAVYYCARDRIIVRPDYFDYWGQGTLLVTVSS
Consensus    RVTMTTDTSTXTAYMELRSLRSDDTAVYYCARDRXXVRPDYFDYWGQGTLLVTVSS

```

**Figure 1D**IgHV4-34-01 / IGHJ4-02 – VH alignment (Group 2, No. 1)

```

IgHV4-34-01  QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR
TH1014-025  QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLKSR
VH1014-001   QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWNWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR
VH1014-143   QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLMR
VH1014-019   QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLKSR
VH1014-021   QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLKSR
VH1014-027   QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYFNNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLKSR
Consensus    QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYFNNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLXSR

IgHV4-34-01  VTISVDTSKNQFSLKLSSVTAADTAVYYCAR-----YFDYWGQGTLLVTVSS
TH1014-025  VTISVDTSKNQFSLKLSSVTAADTAVYYCARGYYDSGVYYFDYWAQGTLLVTVSS
VH1014-001   VTISVDTSKNQFSLKLSSVTAADTAVYYCARGNYGSGYYYFDLWGRGTQVTVSS
VH1014-143   VTISVDTSKNQFSLQLSSVTAADTAVYYCARGYYGSGYYYFDYWGQGTLLVTVSS
VH1014-019   VTISVDTSKNQFSLKLSSVTAADTAVYYCARGYYDSGVYYFDYWAQGTLLVTVSS
VH1014-021   VTISVDTSKNQFSLKLSSVTAADTAVYYCARGYYASGVYYFDYWGQGTLLVTVSS
VH1014-027   VTISVDTSKNQFSLNLSSVTAADTAVYYCARGLIGSGYYYFDYWDQGTLLVTVSS
Consensus    VTISVDTSKXQFSLXLSSVTAADTAVYYCARGXXXSGYYYFDXWXXGTXTVTVSS

```

**Figure 1E**IgHV4-34-01 / IGHJ4-02 – VH alignment (Group 2, No. 2)

IgHV4-34-01 QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYPNPSLKSR  
 TH1014-091 QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYPNPSLKSR  
 VH1014-032 QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYPNPSLKSR  
 VH1014-035 QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYPNPSLKSR  
 VH1014-036 QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYPNPSLKSR  
 VH1014-054 QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYPNPSLKSR  
 VH1014-094 QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYPNPSLKSR  
 Consensus QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYPNPSLKSR

IgHV4-34-01 VTISVDTSKNQFSLKLSSVTAADTAVYYCAR-----YFDYWGQGTLLTVTVSS  
 TH1014-091 VTISVDTSKNQFSLKLSSVTAADTAVYYCARLGGSGYYFDYWGQGTLLTVTVSS  
 VH1014-032 VTISVDTSKNQFSLKLSSVTAADTAVYYCARLGGSGYYFDYWGQGTLLTVTVSS  
 VH1014-035 VTISVDTSKNQFSLKLSSVTAADTAVYYCARLGGSGYYFDYWGQGTLLTVTVSS  
 VH1014-036 VTISVDTSKNQFSLKLSSVTAADTAVYYCARLGGSGYYFDYWGQGTLLTVTVSS  
 VH1014-054 VTISVDTSKNQFSLKLSSVTAADTAVYYCARLGGSGYYFDYWGQGTLLTVTVSS  
 VH1014-094 VTISVDTSKNQFSLKLSSVTAADTAVYYCARLGGSGYYFDYWGQGTLLTVTVSS  
 Consensus VTISVDTSKNQFSLKLSSVTAADTAVYYCARLGGSGYYFDYWGQGTLLTVTVSS

**Figure 1F**IgHV3-30-3-01 / IGHJ4-02 – VH alignment (Group 2)

IgHV1-30-... QVQLVESGGGVVQPGRSLRLSCAASGFTFSYAMHWVRQAPGKGLEWVAVISYDGSNKKYADSVKGR  
 TH1014-129 QVQLVESGGGVVQPGRSLRLSCAASGFTFSYAMHWVRQAPGKGLEWVAVISYDGSNKKYADSVKGR  
 Consensus QVQLVESGGGVVQPGRSLRLSCAASGFTFSYAMHWVRQAPGKGLEWVAVISYDGSNKKYADSVKGR

IgHV3-30-... FTISRDN SKNTLYLQMNSLRAEDTAVYYCAR-----YFDYWGQGTLLTVTVSS  
 TH1014-129 FTISRDN SKNTLYLQMNSLRAEDTAVYYCARGLGVWGAFDYWGQGTLLTVTVSS  
 Consensus FTISRDN SKNTLYLQMNSLRAEDTAVYYCARGLGVWGAFDYWGQGTLLTVTVSS

Figure 1G

IgHV3-23-1 / IGHJ4-02 – VH alignment (Group 3a)

```

IgHV3-23-1  EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMWVRQAPGKGLEWVSAISGSGGSTYYADSVKG
TH1014-098  EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMWVRQAPGKGLEWVSAISGSAYSTYYADSVKG
VH1014-105  EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMWVRQAPGKGLEWVSAISGSAYSTYYADSVKG
VH1014-100  EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMWVRQAPGKGLEWVSAISGSGYSTYYADSVKG
VH1014-125  EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMWVRQAPGKGLEWVSAISGSGYSTYYADSVKG
VH1014-162  EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMWVRQAPGKGLEWVSAISGSGYSTYYADSVKG
Consensus   EVQLLESGGGLVQPGGSLRLSCAASGFTFSNYGMWVRQAPGKGLEWVSAISGSGYSTYYADSVKG

IgHV3-23-1  RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAK-----YFDYWGQGLTVTVSS
TH1014-098  RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKHYHSGSGSYTTFDYWGQGLTVTVSS
VH1014-105  RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKHYHSGSGSYTTFDYWGQGLTVTVSS
VH1014-100  RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKHYHSGSGSYTTFDYWGQGLTVTVSS
VH1014-125  RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKHYHSGSGSYTTFDYWGQGLTVTVSS
VH1014-162  RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKHYHSGSGSYTTFDYWGQGLTVTVSS
Consensus   RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAKHYHSGSGSYTTFDYWGQGLTVTVSS

```

Figure 1H

IgHV5-51-01 / IGHJ2-01 – VH alignment (Group 3a, No. 1)

```

IgHV5-51-01  EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQG
TH1014-127   EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQG
Consensus    EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQG

IgHV5-51-01  QVTISADKSISTAYLQWSSLKASDTAMYYCAR-----YFDLWGRGTLTVTVSS
TH1014-127   QVTISADKSISTAYLQWSSLKASDTAMYYCARQPGDWS PRHWYFDLWGRGTLTVTVSS
Consensus     QVTISADKSISTAYLQWSSLKASDTAMYYCARQPGDWS PRHWYFDLWGRGTLTVTVSS

```

Figure 1I

IgHV5-51-01-01 / IGHJ5-02 – VH alignment (Group 3a, No. 2)

```

IgHV5-51-01  EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQG
TH1014-159   EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQG
Consensus     EVQLVQSGAEVKKPGESLKISCKGSGYSFTSYWIGWVRQMPGKGLEWMGIIYPGDS DTRYSPSFQG

IgHV5-51-01  QVTISADKSISTAYLQWSSLKASDTAMYYCAR-----NWFDPWGQGLTVTVSS
TH1014-159   QVTISADKSISTAYLQWSSLKASDTAMYYCARWGTY YDILTGYFNWFDPWGQGLTVTVSS
Consensus     QVTISADKSISTAYLQWSSLKASDTAMYYCARWGTY YDILTGYFNWFDPWGQGLTVTVSS

```

Figure 1J

IgHV1-18-01 / IGHJ6-02 – VH alignment (Group 3b)

IgHV1-18-01 QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQG  
**TH1014-132** QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGN~~SNYVQK~~QG  
 Consensus QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGISWVRQAPGQGLEWMGWISAYNGN~~SNYVQK~~QG  
  
 IgHV1-18-01 RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAR-----GMDVWGQGTITVTVSS  
**TH1014-132** RVTMTTDT~~ST~~STAYMELRSL~~RS~~DDTAVYYCAREYSYDSGTYFYYGMDVWGQGTITVTVSS  
 Consensus RVTMTTDTSTSTAYMELRSLRSDDTAVYYCAREYSYDSGTYFYYGMDVWGQGTITVTVSS

Figure 1K

IgHV3-30-3-01 / IGHJ4-02 – VH alignment (Group 3b)

IgHV3-30... QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKG  
**TH1014-153** QVQLVESGGGVVQPGRSLRLSCAASGFTFSS~~Y~~YAMHWVRQAPGKGLEWV~~AV~~ISYDGSNKYYADSVKG  
 VH1014-033 QVQLVESGGGVVQ~~Y~~GRSLRLSCAASGFTFSS~~Y~~AMHWVRQAPGKGLEWV~~AV~~ISYDGSNKYYADSVKG  
 VH1014-160 QVQLVESGGGVVQPGRSLRLSCAASGFTFSS~~Y~~AMHWVRQAPGKGLEWV~~AV~~ISYDGSNKYYADSVKG  
 VH1014-166 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGSN~~E~~YYADSVKG  
 VH1014-152 QVQVVESGGGVVQPGRSLRLSCAASGFTFSSYAMHWVRQAPGKGLEWVAVISYDGS~~N~~KYYADSVKG  
 VH1014-167 QVQLVESGGGVVQPGRSLRLSCAASGFTFSSY~~A~~AMHWVRQAPGKGLEWVAVISYDGSNKYYADSVKG  
 Consensus QVQLVESGGGVVQ~~Y~~GRSLRLSCAASGFTFSS~~Y~~~~XXXX~~AMHWVRQAPGKGLEWV~~AV~~ISYDGS~~N~~~~XXXX~~YYADSVKG  
  
 IgHV3-30... RFTISRDN SKNTLYLQMNSLRAEDTAVYYCAR-----~~F~~FDYWGQGTITVTVSS  
**TH1014-153** RFTISRDN SKNTLYLQMNSL~~RA~~EDTAVYYCARG~~ST~~IGVFDYWGQGTITVTVSS  
 VH1014-033 RFTISRDN SKNTLYLQMNSLRAEDTAVYYCARG~~ST~~IGVFDYWGQGTITVTVSS  
 VH1014-160 RFTISRDN SKNT~~MY~~LQMNSLRAEDTAVYYCARG~~ST~~IGVFDYWGQGTITVTVSS  
 VH1014-166 RFTISRDN SKNTLYLQMNSLRAEDTAVYYCARG~~ST~~IGVFDYWGQGTITVTVSS  
 VH1014-152 RFTISRDN SKNTLYLQMNSLRAEDTAVYYCARG~~ST~~IGVFDYWGQGTITVTVSS  
 VH1014-167 RFTISRDN SKNTLYLQMNSLRAEDTAVYYCARG~~ST~~IGVFDYWGQGTITVTVSS  
 Consensus RFTISRDN SKNT~~MY~~LQMNSL~~RA~~EDTAVYYCARG~~ST~~~~XXXXXX~~IGVFDYWGQGTITVTVSS

**Figure 2A**IgKV1-12-01 / IGKJ5-01 – VL alignment (Group 1)

IgKV1-12-01 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQOKPGKAPKLLIYAASSLQSGVPSRFSGSG  
 VL1014-050 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQOKPGKAPKLLIYAASLQSGVPSRFSGSG  
 VL1014-084 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQOKPGKAPKLLIYASLQSGVPSRFSGSG  
 VL1014-049 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQOKPGKAPKLLIYAASLQSGVPSRFSGSG  
 VL1014-051 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQOKPGKAPKLLIYAASLQSGVPSRFSGSG  
 VL1014-055 DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQOKPGKAPKLLIYAASLQSGVPSRFSGSG  
 Consensus DIQMTQSPSSVSASVGDRVTITCRASQGISSWLAWYQOKPGKAPKLLIYASLQSGVPSRFSGSG  
  
 IgKV1-12-01 SGTDFTLTISSLPEDFATYYCQQANSFPITFGQGRLEIK  
 VL1014-050 SGTDFTLTISSLPEDFATYYCQQANSFPITFGQGRLEIK  
 VL1014-084 SGTDFTLTISSLPEDFATYYCQQANSFPITFGGGRLEIK  
 VL1014-049 SGTDFTLTISSLPEDFATYYCQQANSFPITFGQGRLEIK  
 VL1014-051 SGTDFTLTISSLPEDFATYYCQQANSFPITFGQGRLEIK  
 VL1014-055 SGTDFTLTISSLPEDFATYYCQQANSFPITFGQGRLEIK  
 Consensus SGTDFTLTISSLPEDFATYYCQQANSFPITFGGGRLEIK

**Figure 2B**IgKV3-11-01 / IGKJ1-01 – VL alignment (Group 1)

IgKV3-11-01 EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQOKPGQAPRLLIYDASNRTGIPARFSGSG  
 VL1014-169 EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQOKPGQAPRLLIYDASNRTGIPARFSGSG  
 VL1014-124 EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQOKPGQAPRLLIYDASNRTGIPARFSGSG  
 VL1014-161 EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQOKPGQAPRLLIYDASNRTGIPARFSGSG  
 VL1014-123 EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQOKPGQAPRLLIYDASNRTGIPARFSGSG  
 Consensus EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQOKPGQAPRLLIYDASNRTGIPARFSGSG  
  
 IgKV3-11-01 SGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK  
 VL1014-169 SGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK  
 VL1014-124 SGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK  
 VL1014-161 SGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK  
 VL1014-123 SGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK  
 Consensus SGTDFTLTISSLEPEDFAVYYCQQRSNWPRTFGQGTKVEIK

**Figure 2C**IgKV1D-16-01 / IGKJ5-01– VL alignment (Group 2, No. 1)

```

IgKV1D-16 ... DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-025  DIQMTQSPSSLSASVGDRVTITCRASQGISRWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-001  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-019  DIQMTQSPSSLSASVGDRVTITCRASQGISRWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-143  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-021  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-027  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
Consensus   DIQMTQSPSSLSASVGDRVTITCRASQGISRWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG

IgKV1D-16 ... SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-025  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-001  SGTDFTLTISSLQPEDFATYYCQQYISRPITFGQGTRLEIK
VL1014-019  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-143  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-021  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-027  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
Consensus   SGTDFTLTISSLQPEDFATYYCQQYNSRPITFGQGTRLEIK

```

**Figure 2D**IgKV1D-16-01 / IGKJ1-01– VL alignment (Group 2, No. 2)

```

IgKV1D-16 ... DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-091  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-032  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-035  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-036  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-054  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-094  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
Consensus   DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG

IgKV1D-16 ... SGTDFTLTISSLQPEDFATYYCQQYNSYPWTFGQGTKVEIK
VL1014-091  SGTDFTLTISSLQPEDFATYYCQQYNSFPPTFGQGTKVEIK
VL1014-032  SGTDFTLTISSLQPEDFATYYCQQYNSFPPTFGQGTKVEIK
VL1014-035  SGTDFTLTISSLQPEDFATYYCQQYNSFPPTFGQGTKVEIK
VL1014-036  SGTDFTLTISSLQPEDFATYYCQQYNSFPPTFGQGTKVEIK
VL1014-054  SGTDFTLTISSLQPEDFATYYCQQYNSFPPTFGQGTKVEIK
VL1014-094  SGTDFTLTISSLQPEDFATYYCQQYNSFPPTFGQGTKVEIK
Consensus   SGTDFTLTISSLQPEDFATYYCQQYNSFPPTFGQGTKVEIK

```

Figure 2E

IgKV1D-16-01 / IGKJ2-01 – VL alignment (Group 3a)

```

IgKV1D-16 ... DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-098  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-100  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-105  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-125  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-162  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
Consensus   DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG

IgKV1D-16 ... SGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK
VL1014-098  SGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK
VL1014-100  SGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK
VL1014-105  SGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK
VL1014-125  SGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK
VL1014-162  SGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK
Consensus   SGTDFTLTISSLQPEDFATYYCQQYNSYPYTFGQGTKLEIK

```

Figure 2F

IgKV1D-16-01 / IGKJ5-01 – VL alignment (Group 3b)

```

IgKV1D-16 ... DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-153  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-152  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-166  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-167  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-160  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
VL1014-033  DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG
Consensus   DIQMTQSPSSLSASVGDRVTITCRASQGISSWLAWYQQKPEKAPKSLIYAASSLQSGVPSRFSGSG

IgKV1D-16 ... SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-153  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-152  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-166  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-167  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-160  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
VL1014-033  SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK
Consensus   SGTDFTLTISSLQPEDFATYYCQQYNSYPITFGQGTRLEIK

```

Figure 3A

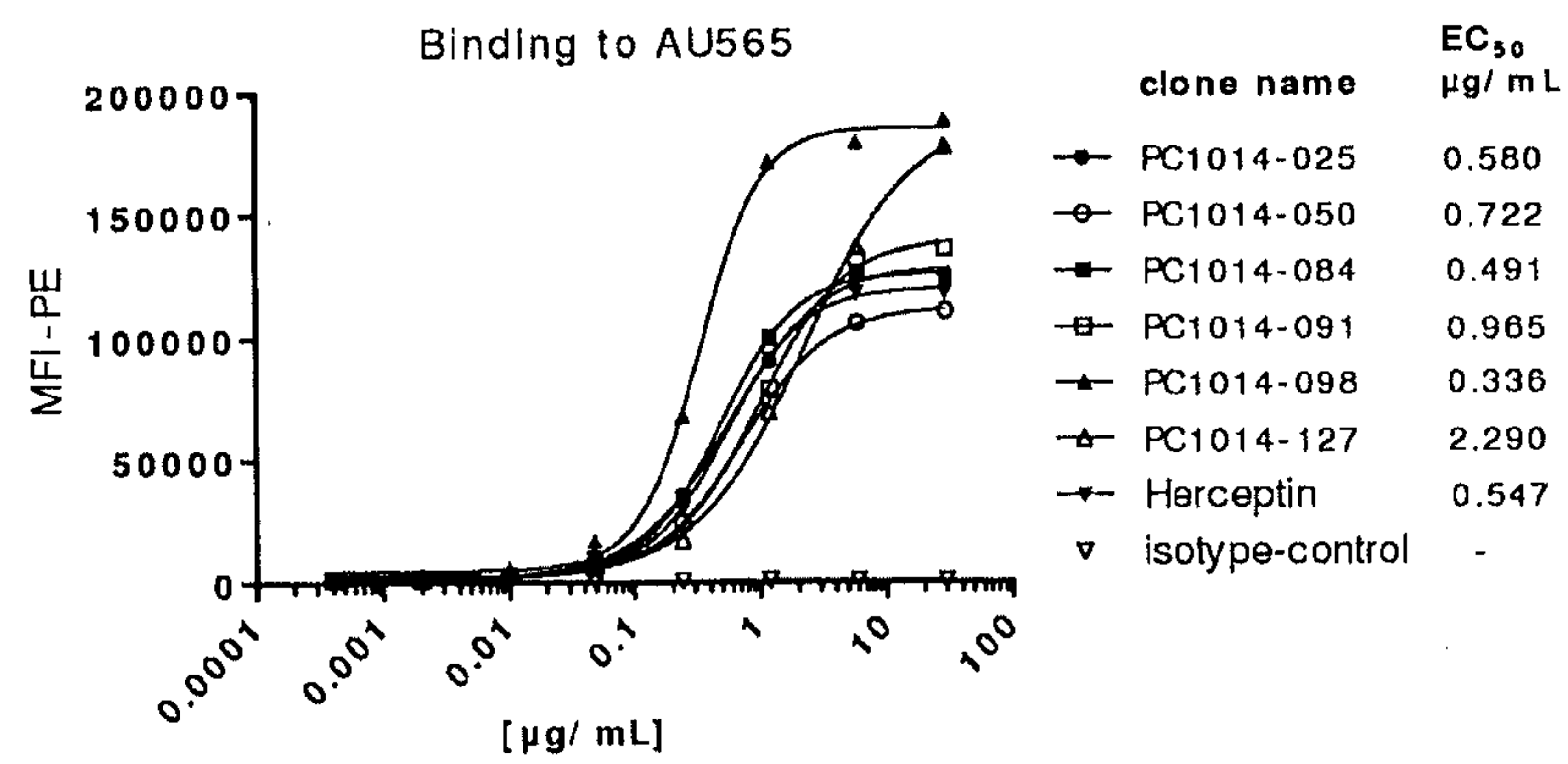


Figure 3B

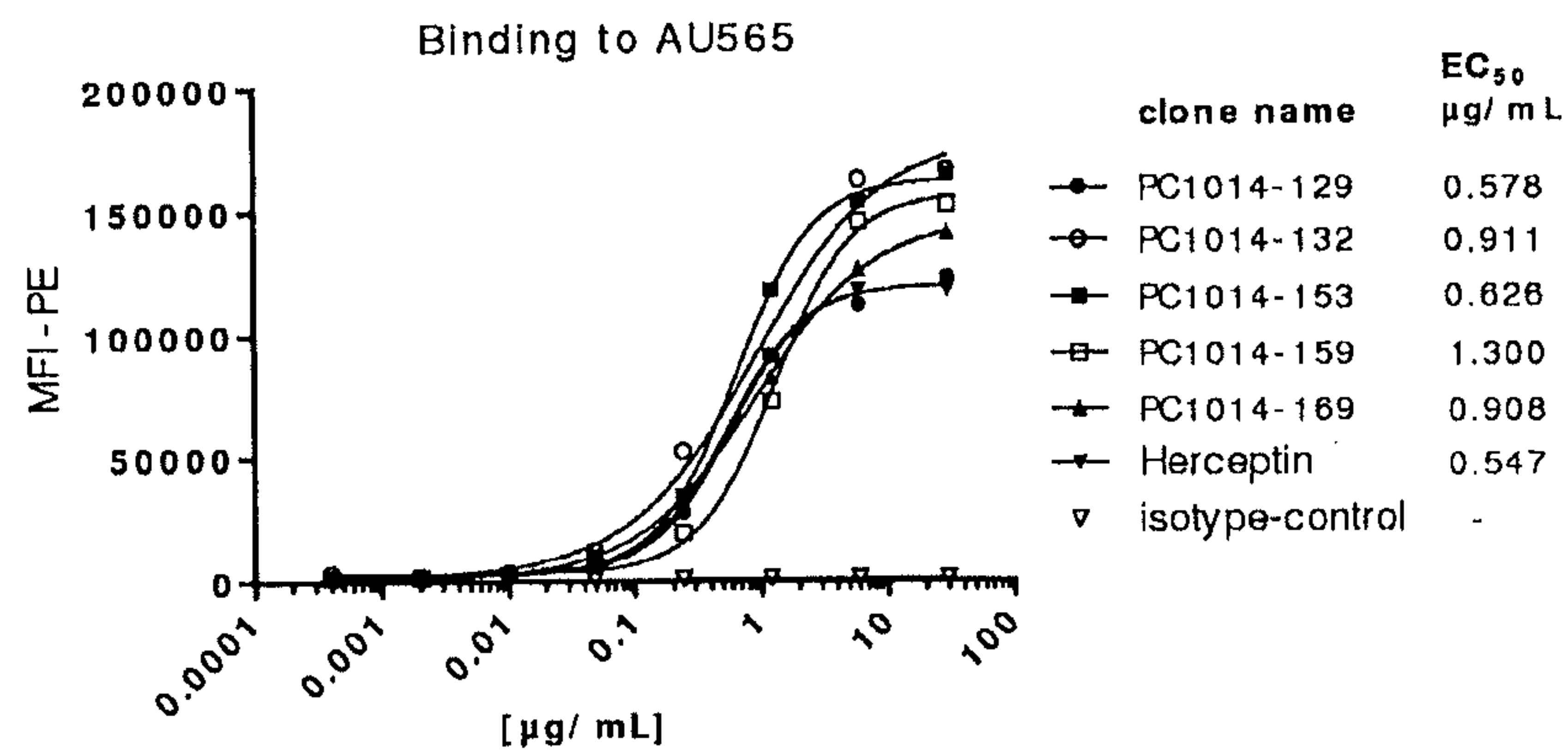


Figure 3C

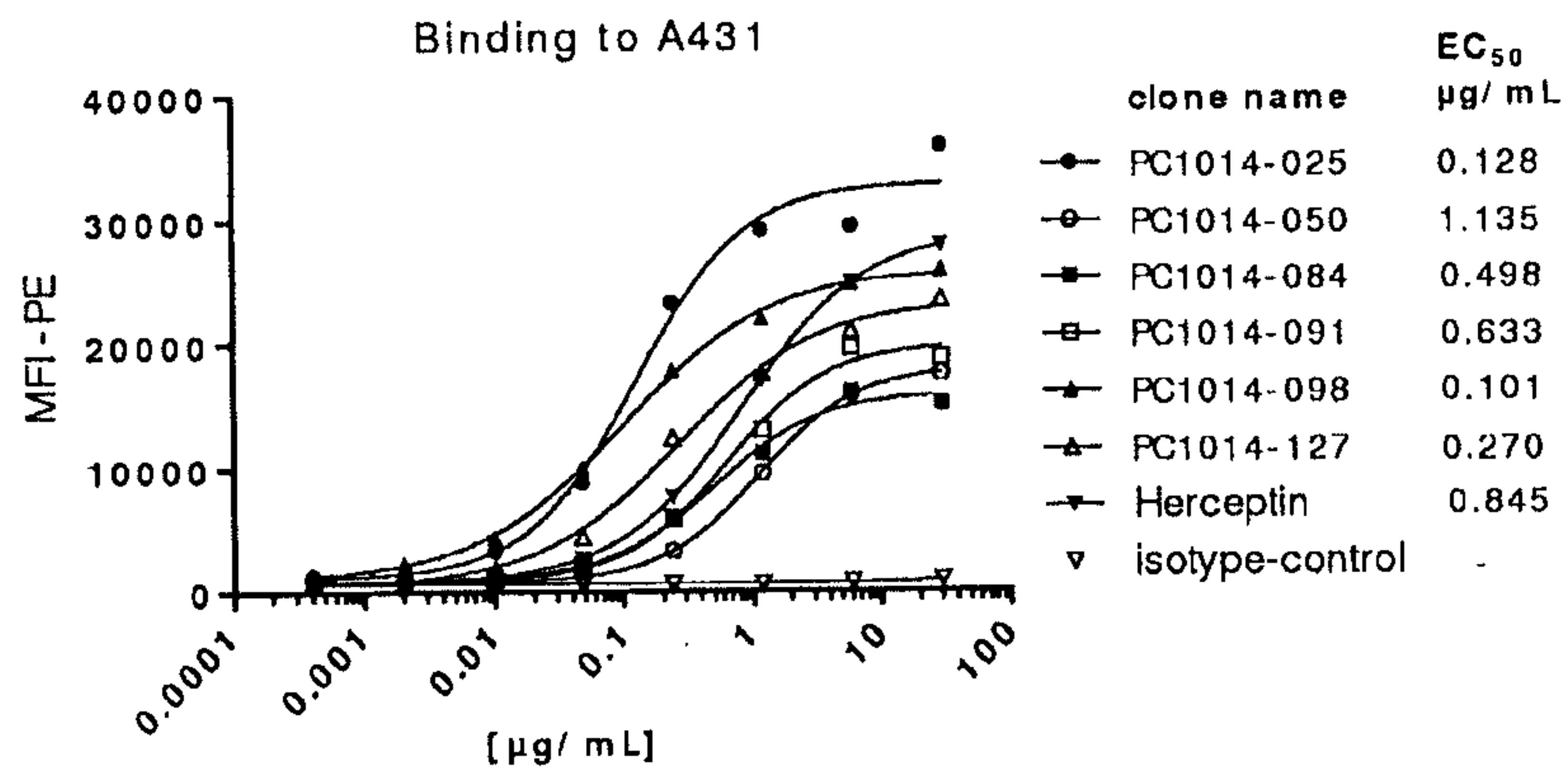


Figure 3D

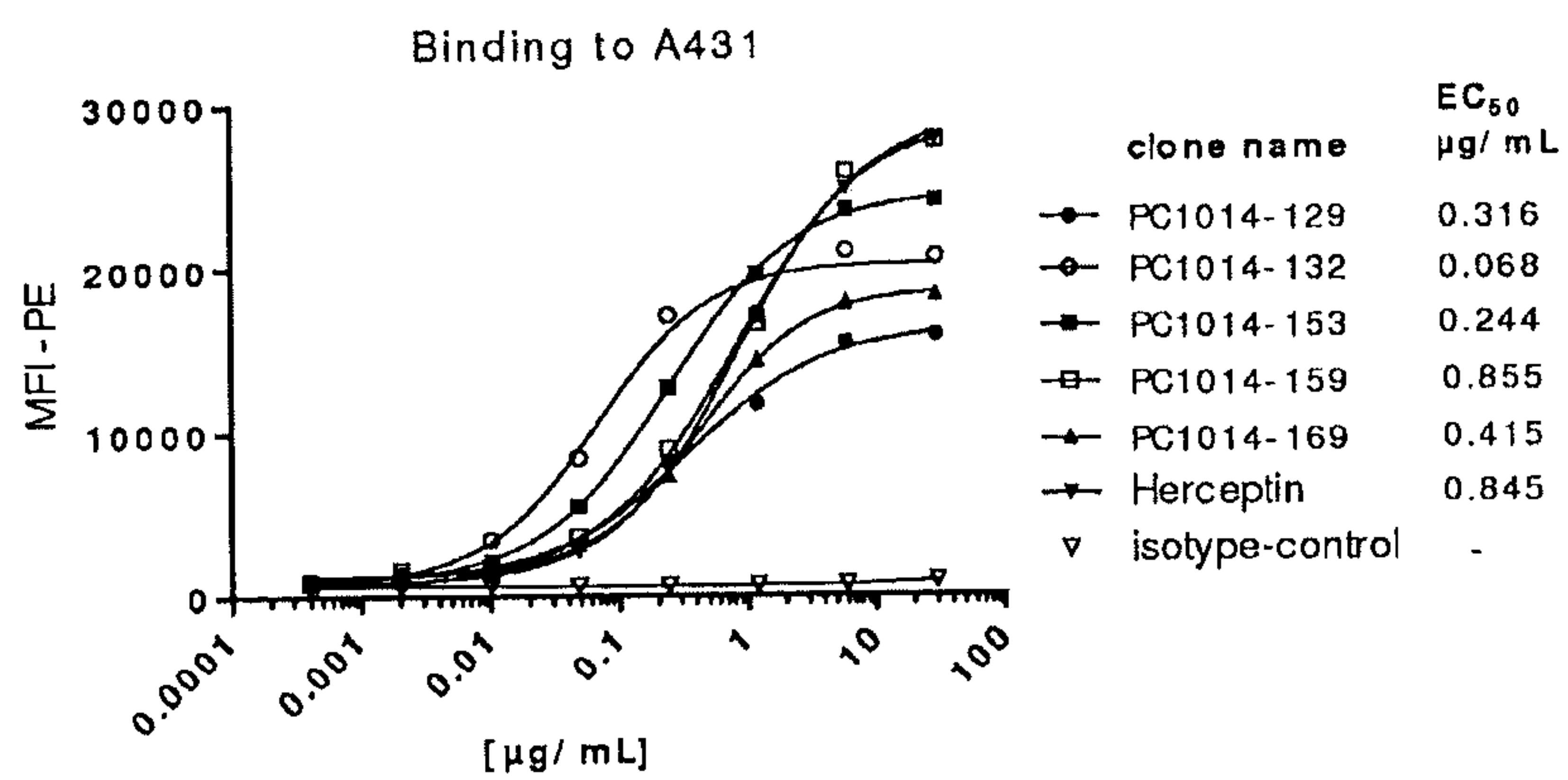


Figure 4

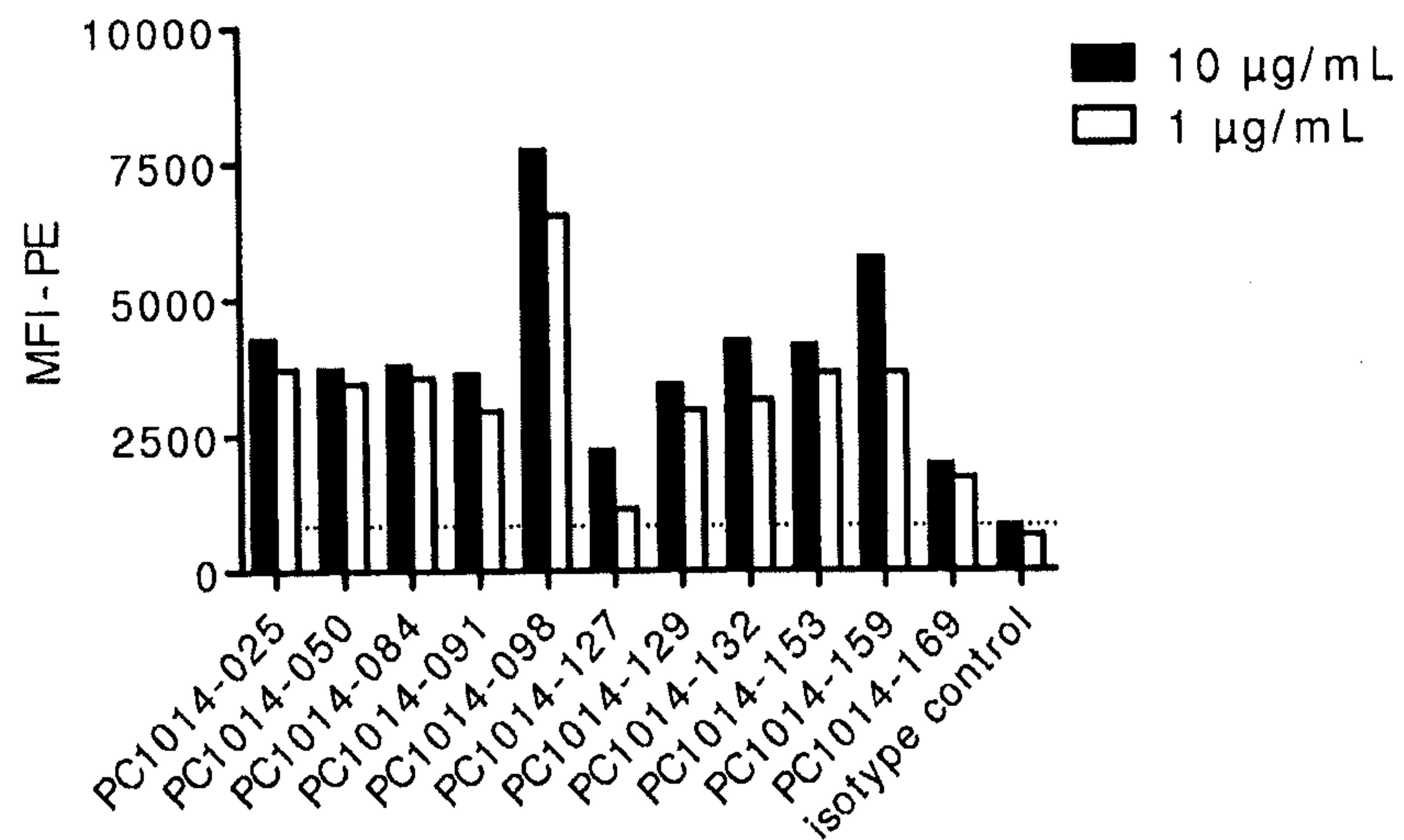


Figure 5

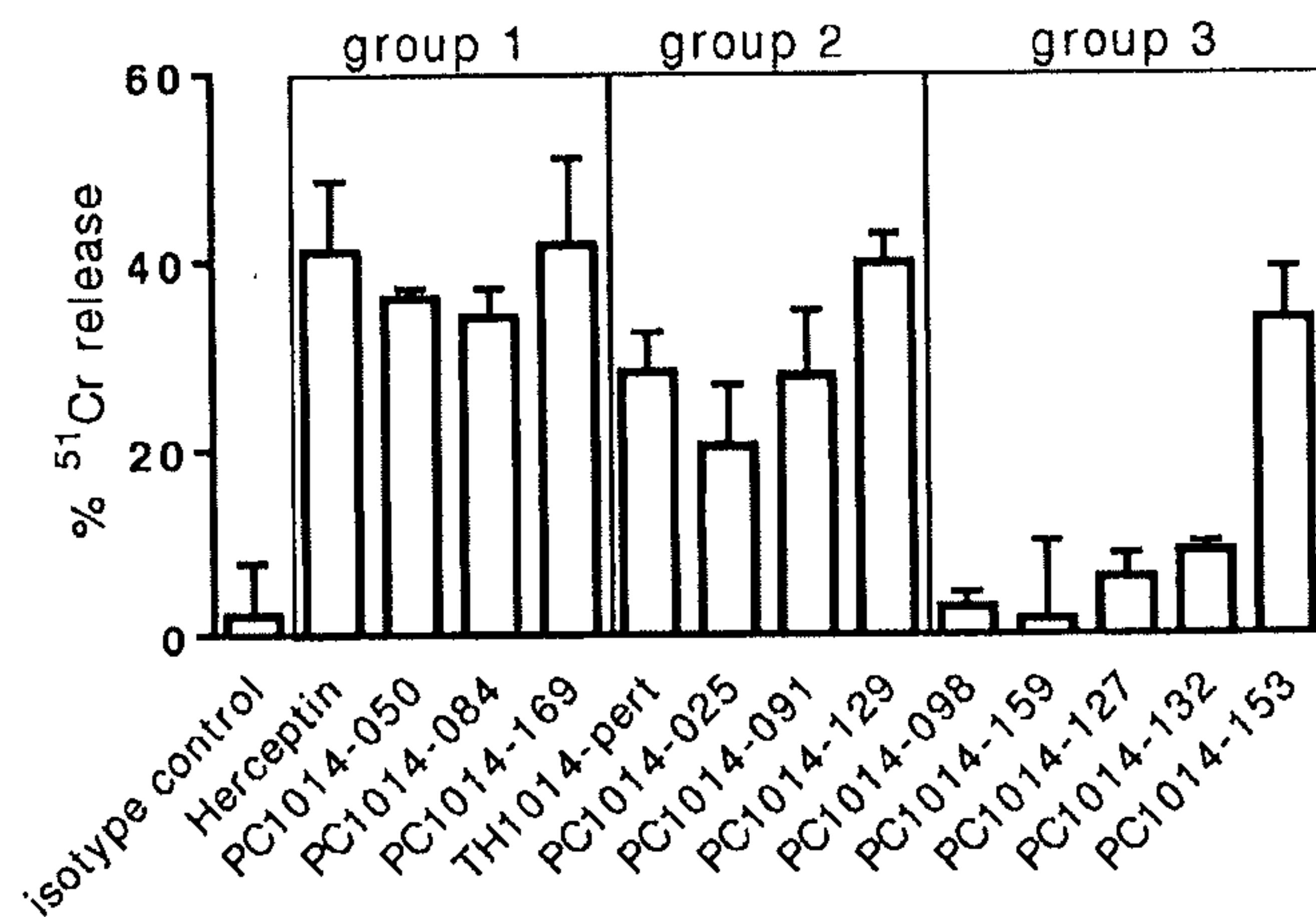


Figure 6

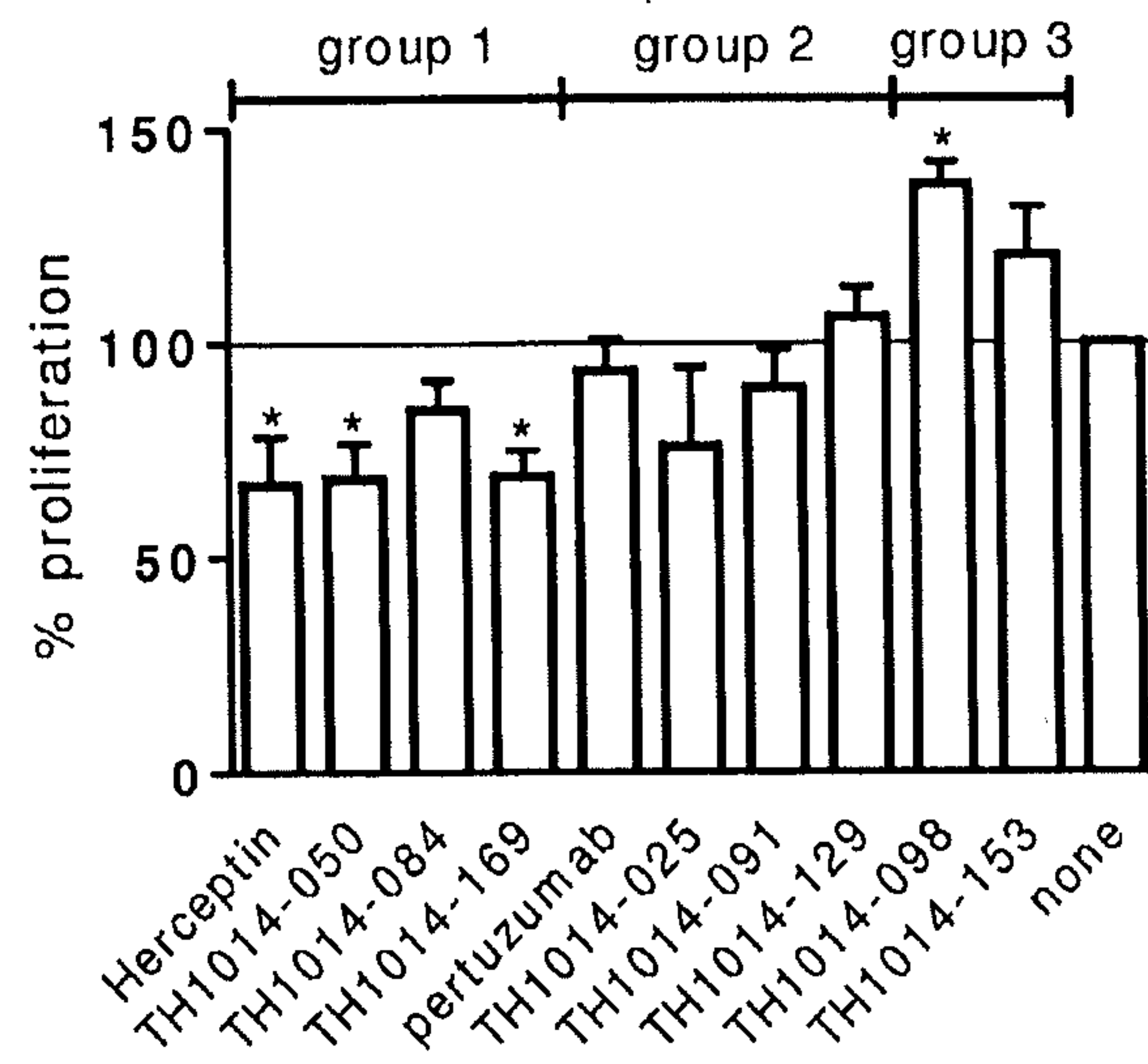


Figure 7

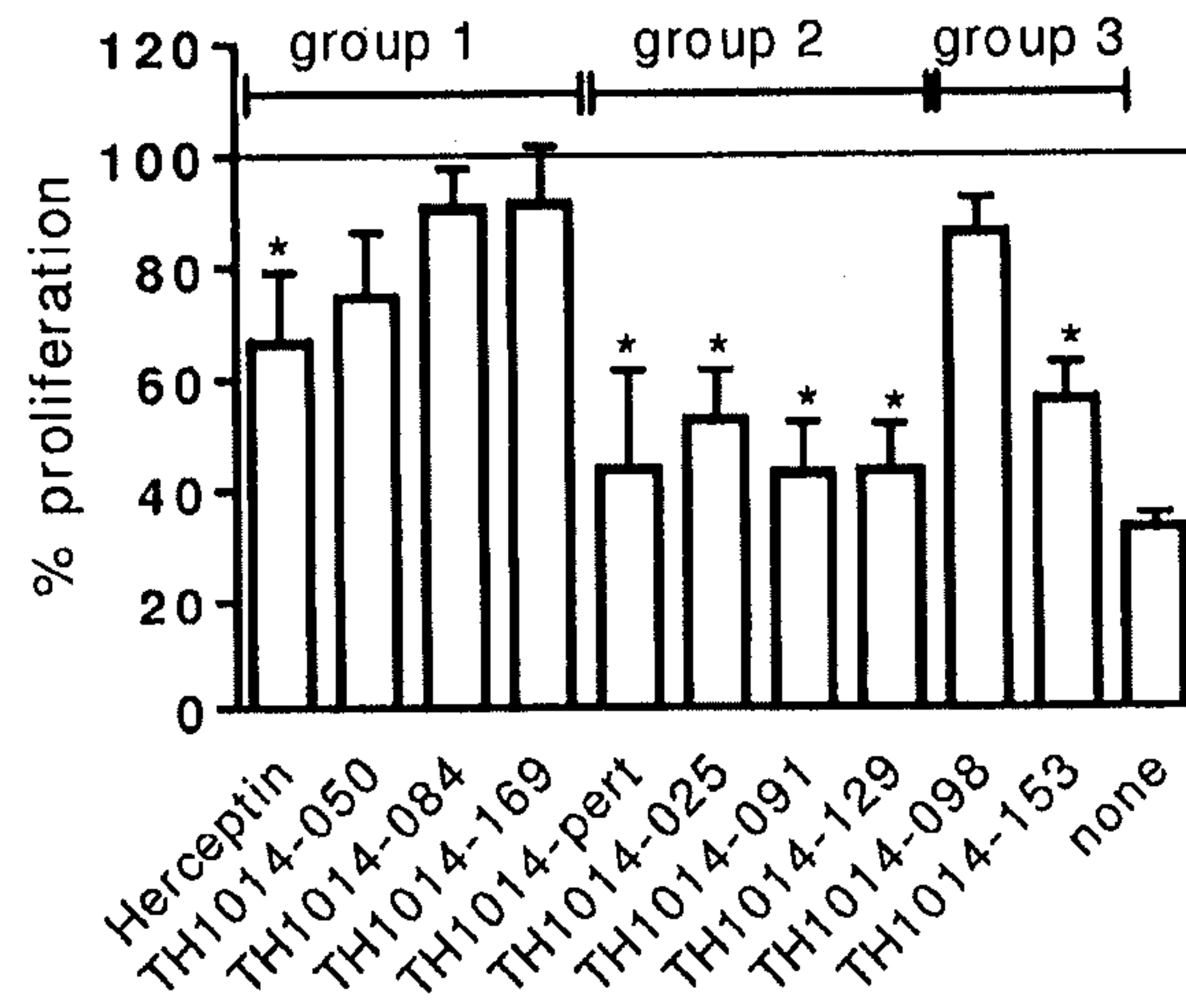


Figure 8A

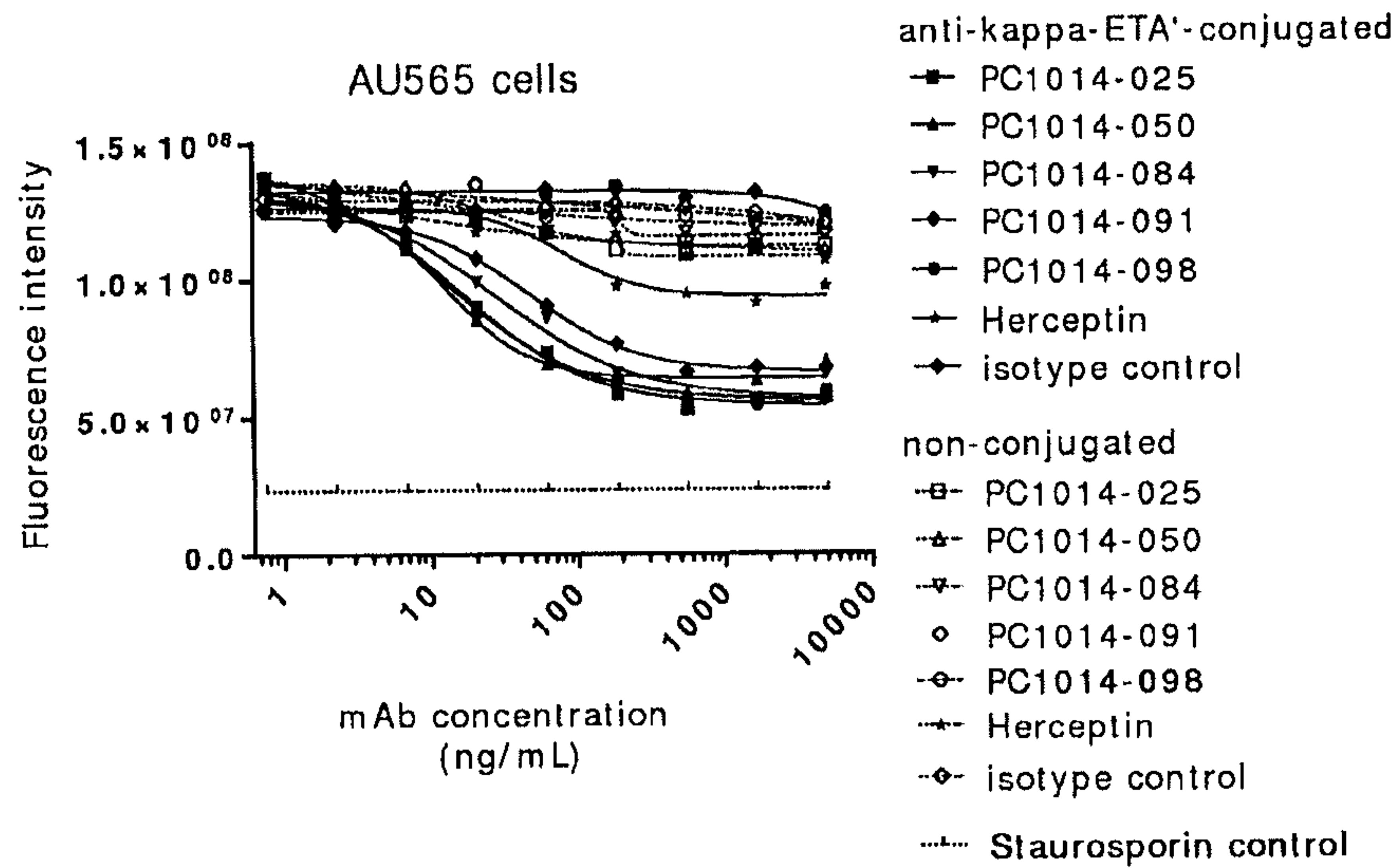


Figure 8B

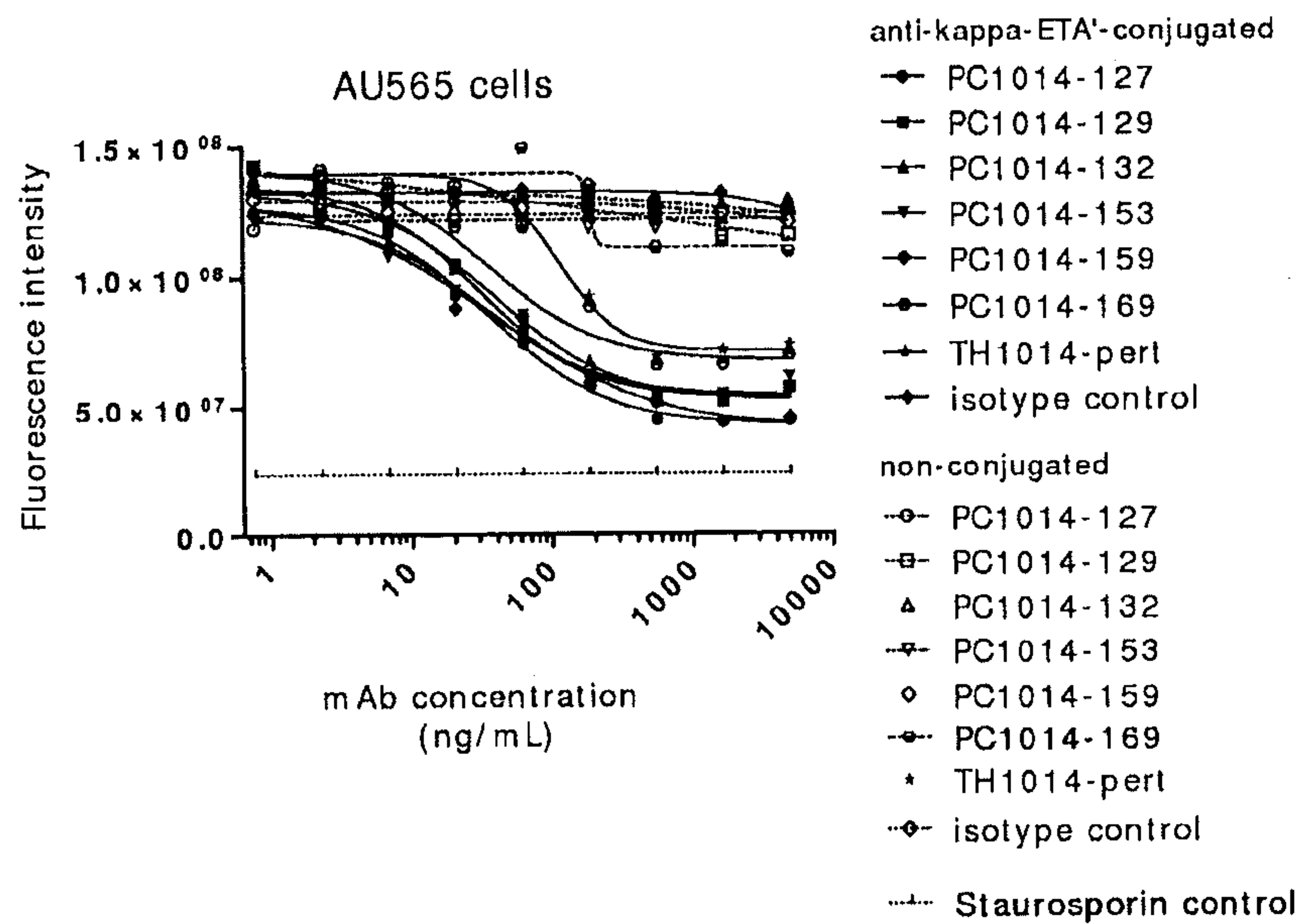


Figure 8C

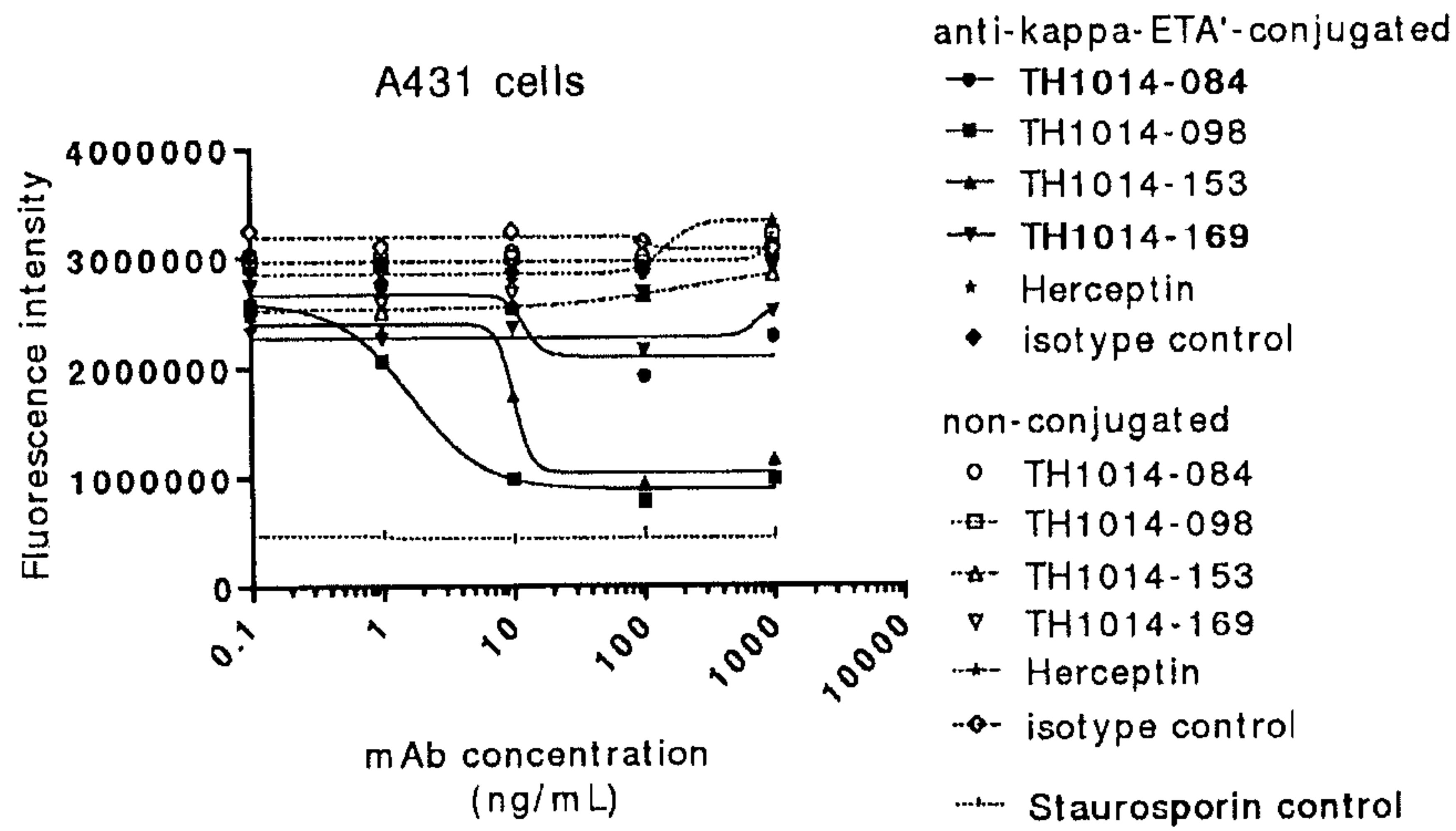


Figure 8D

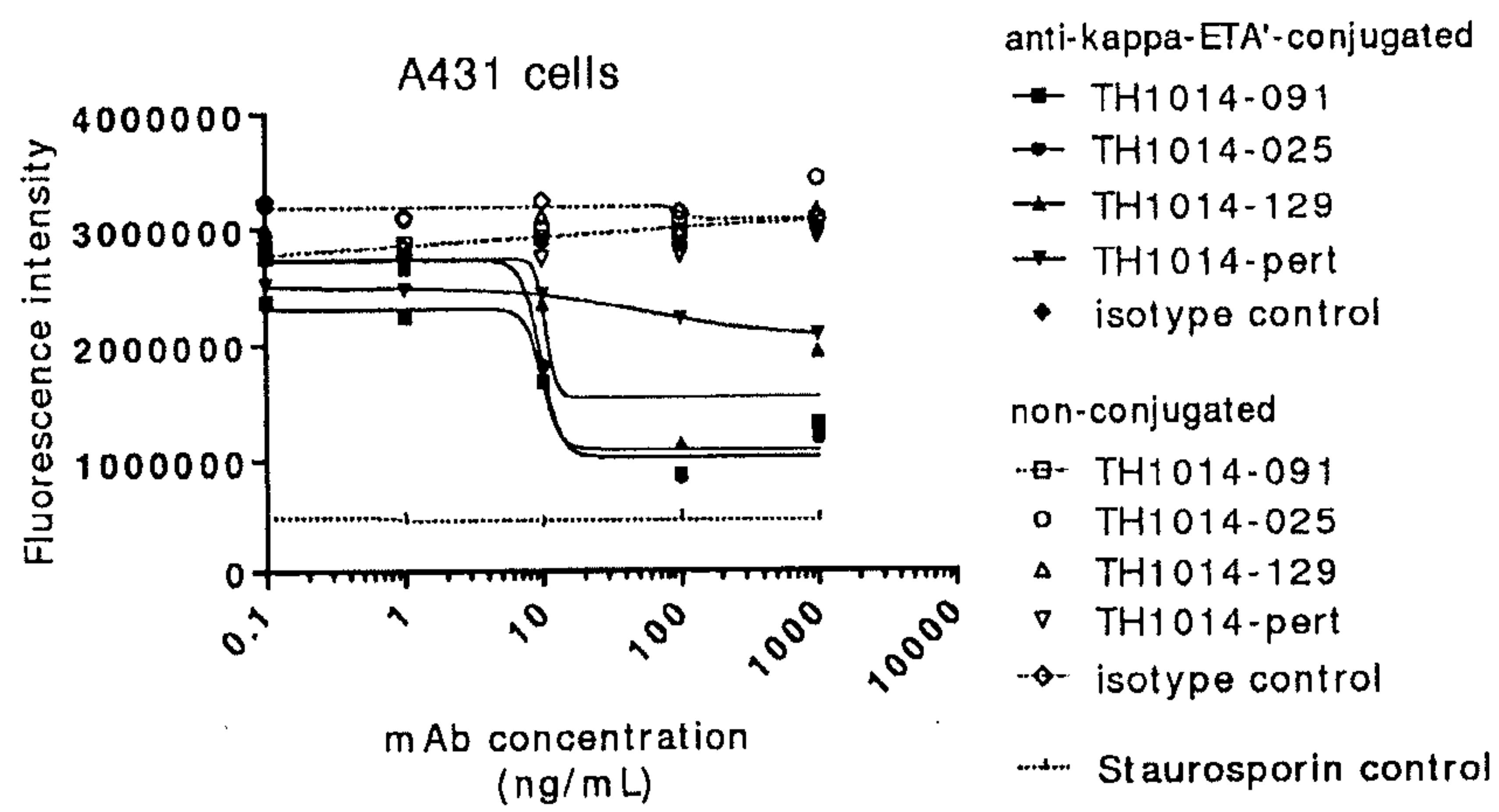


Figure 9

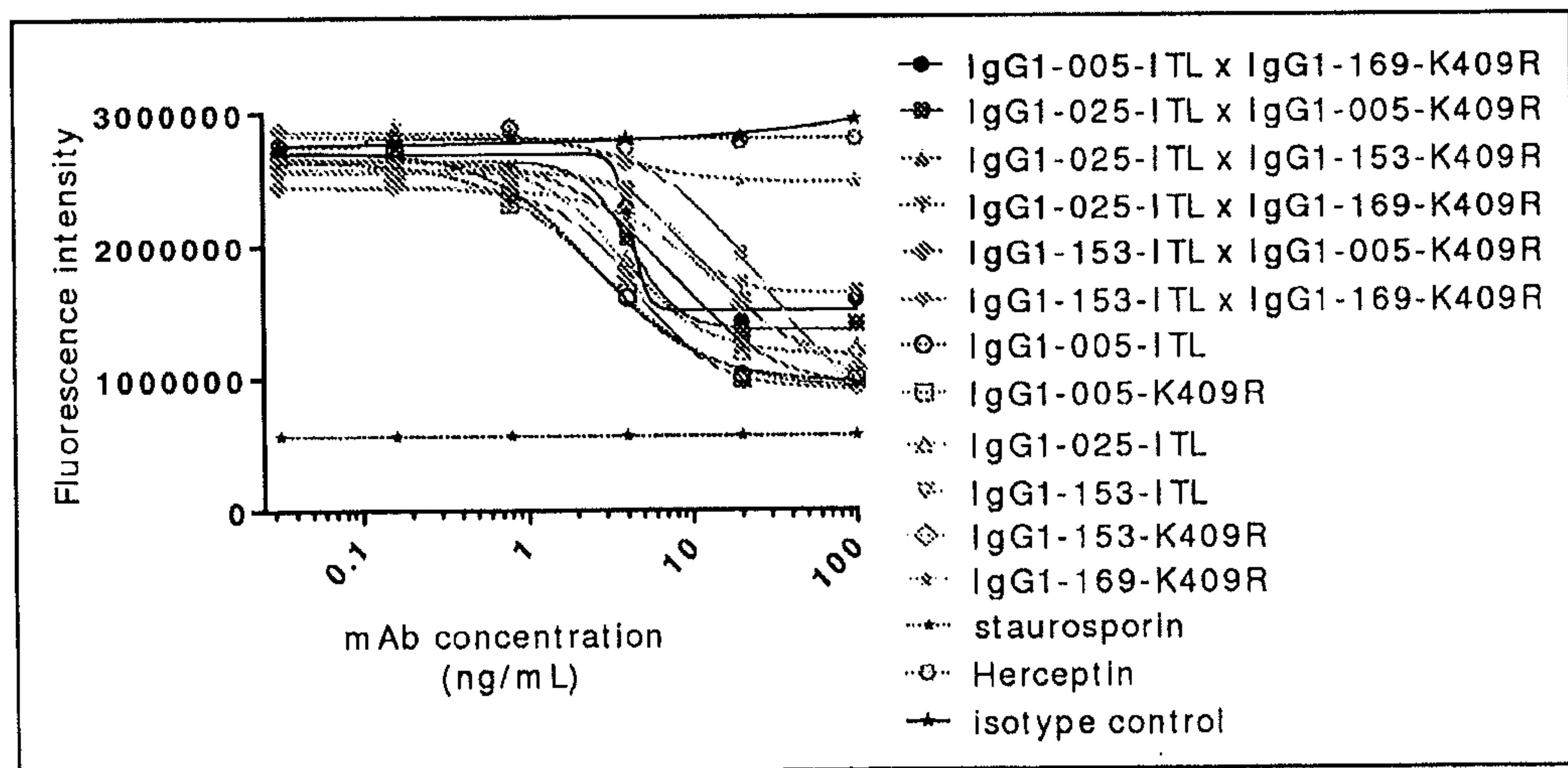


Figure 10

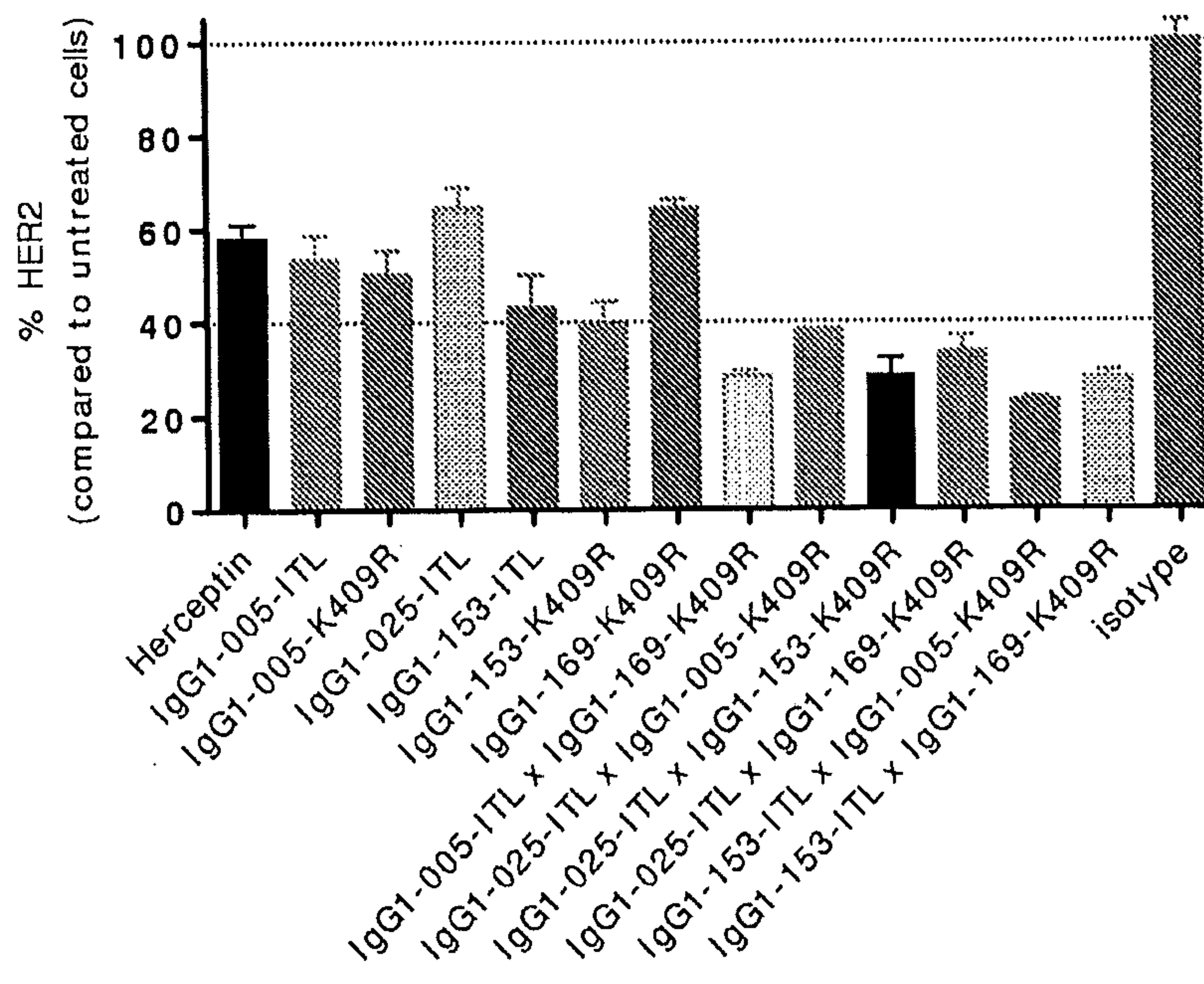
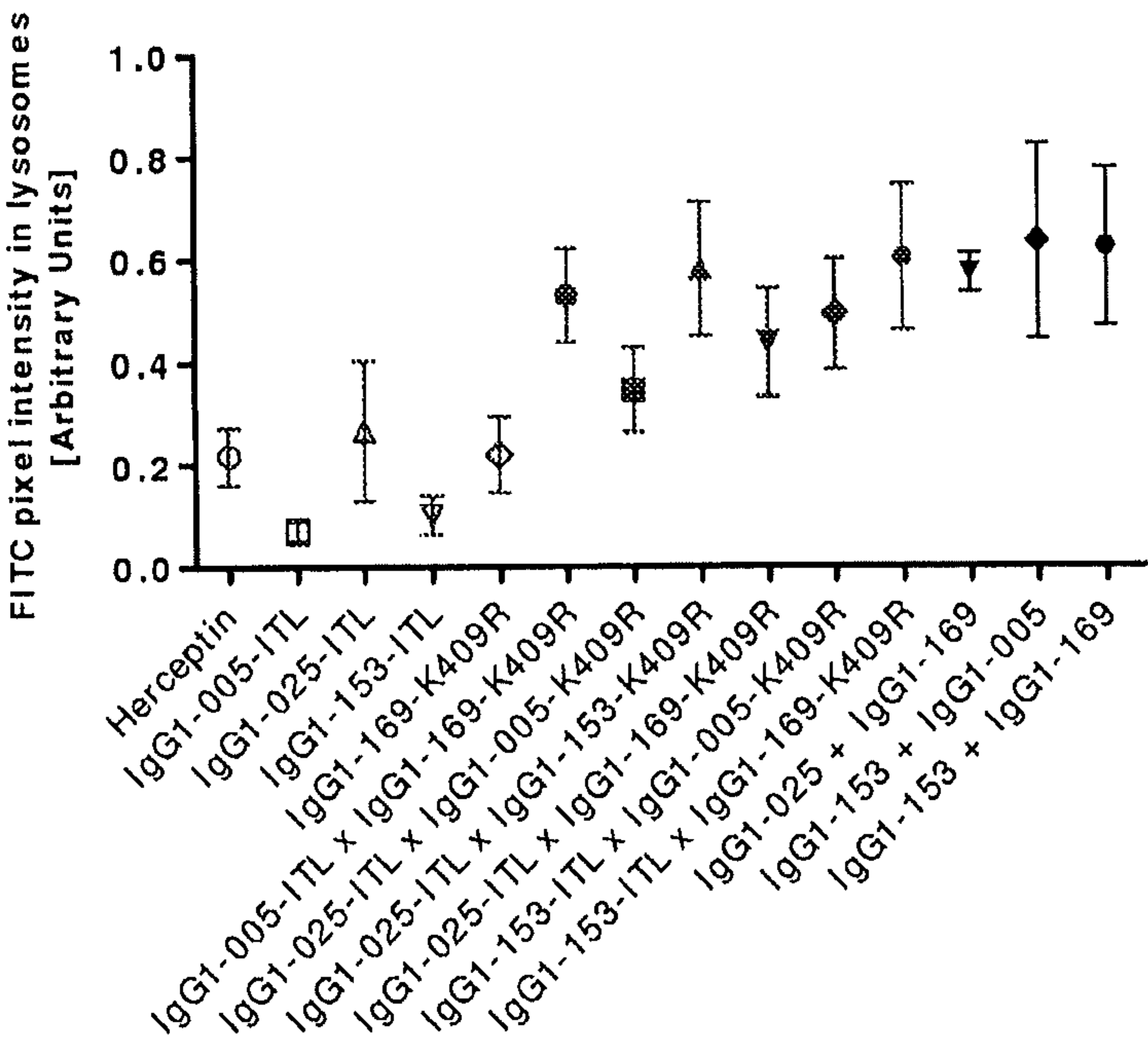


Figure 11

A



B

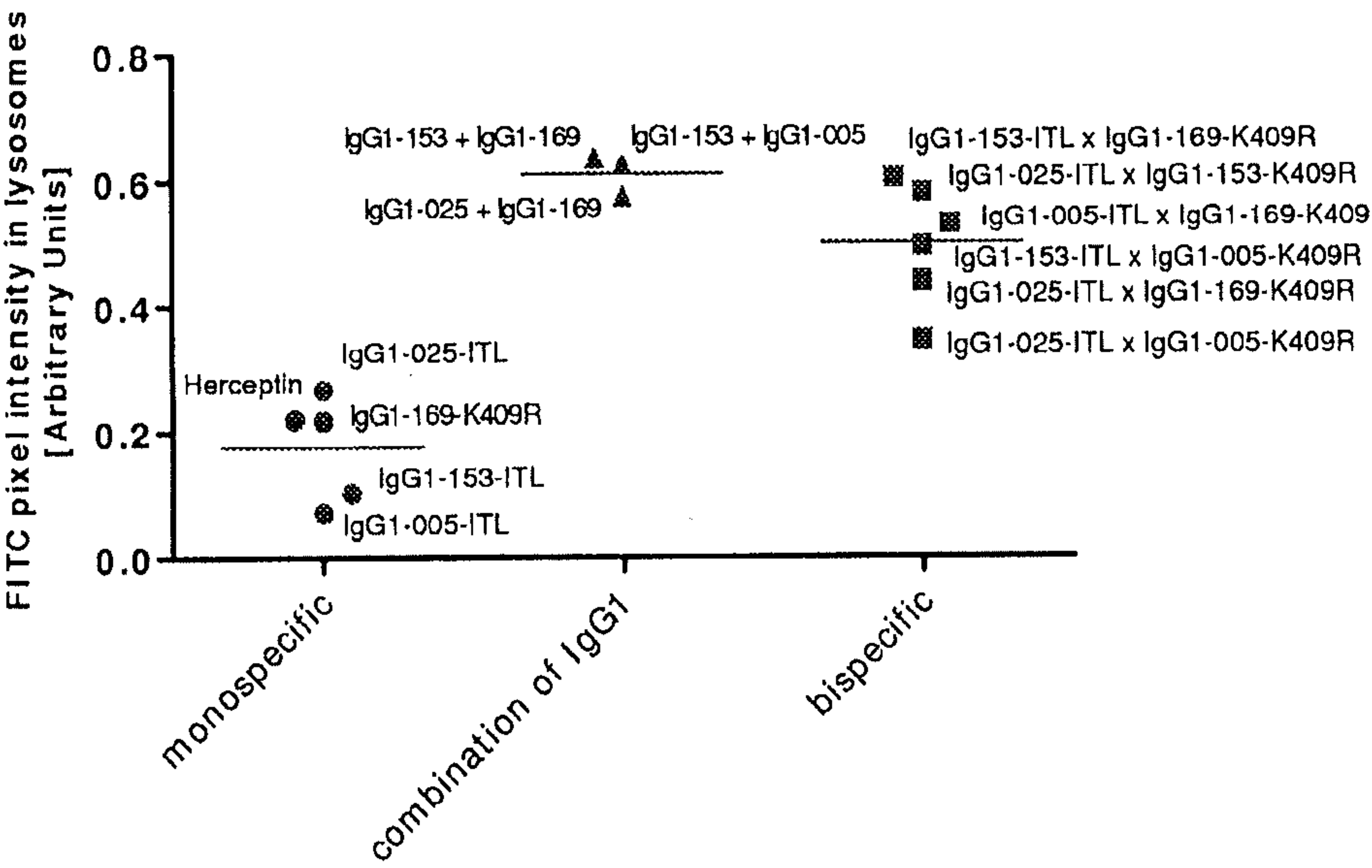


Figure 12

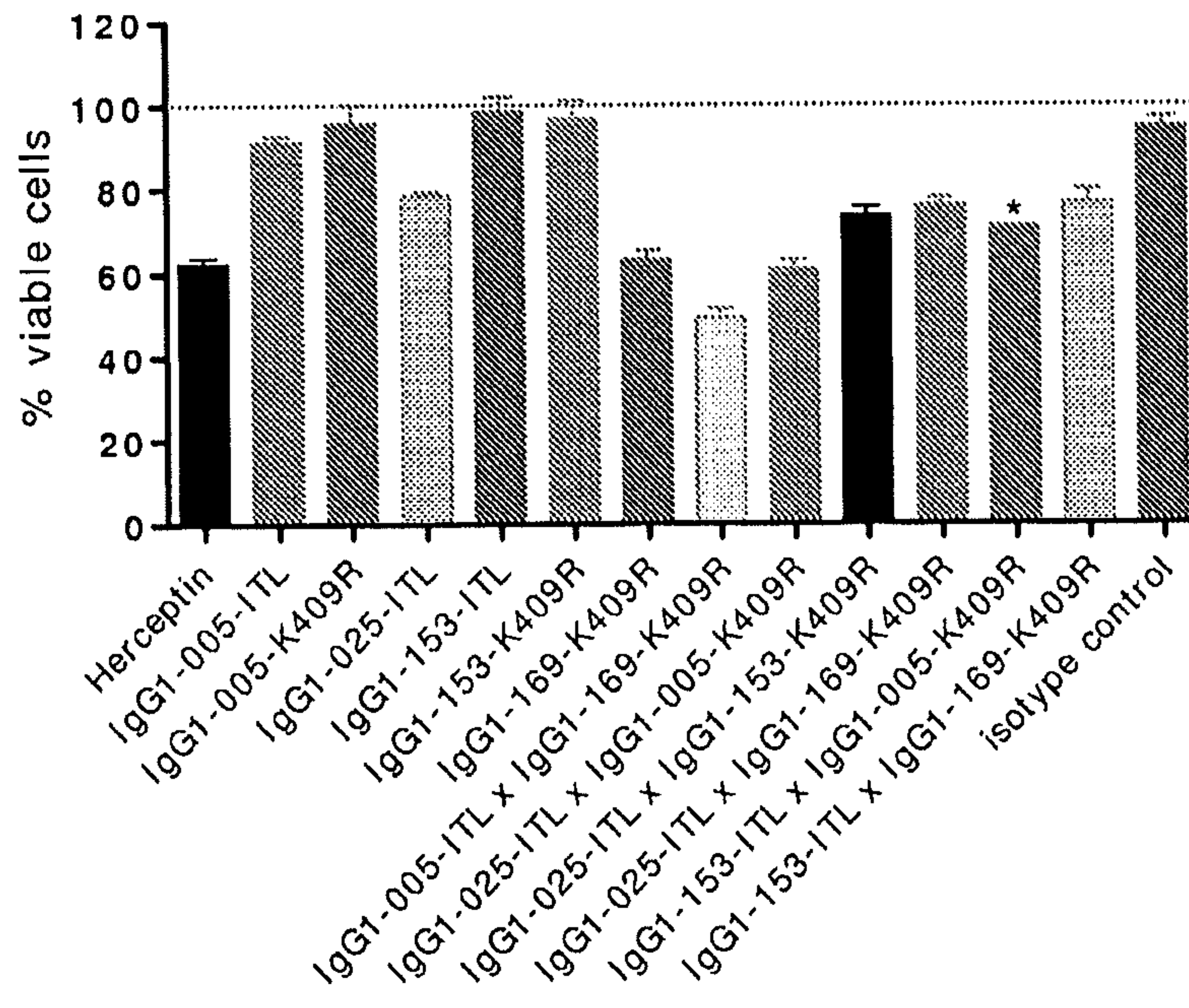


Figure 13

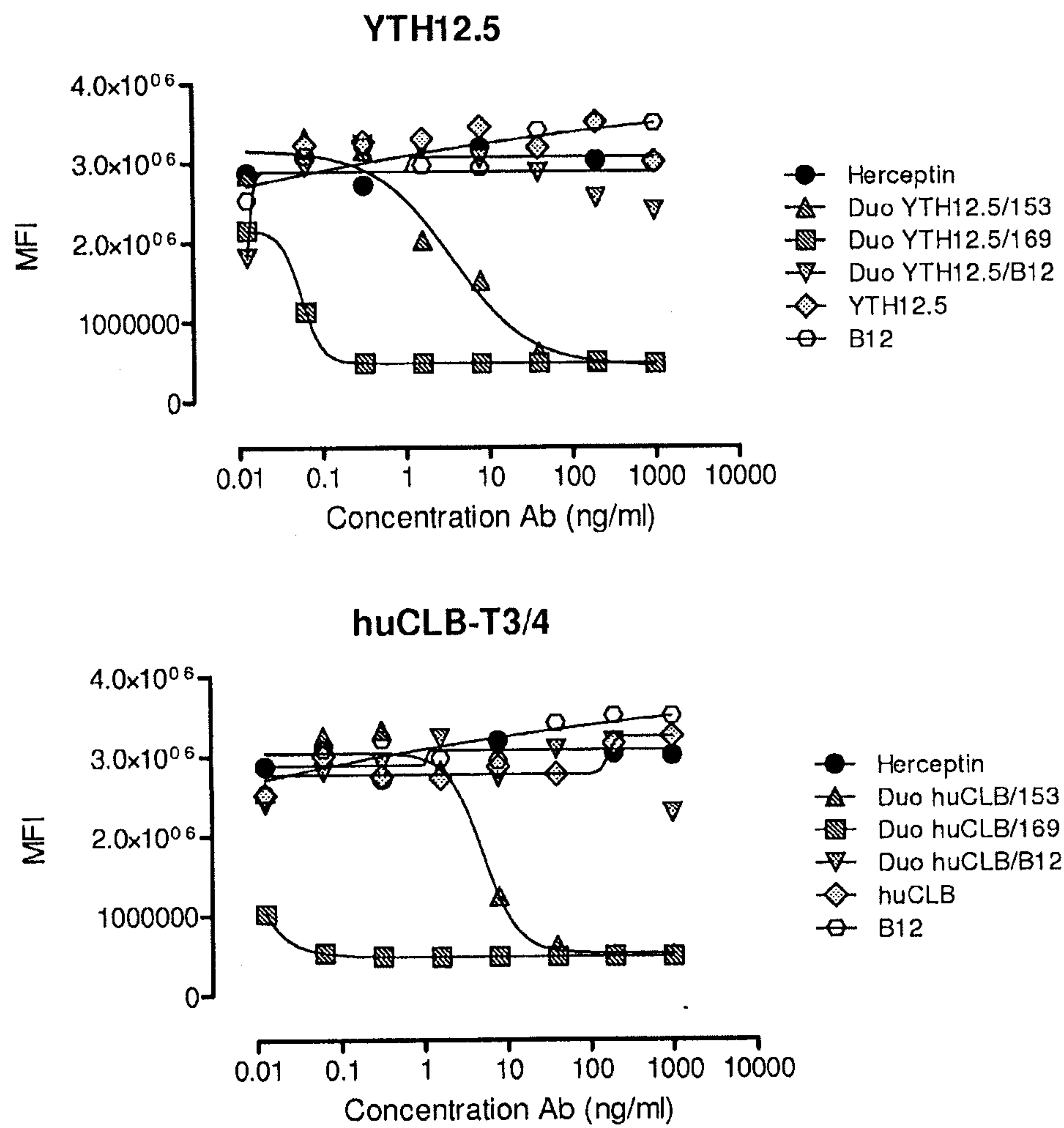


Figure 13 (continued)

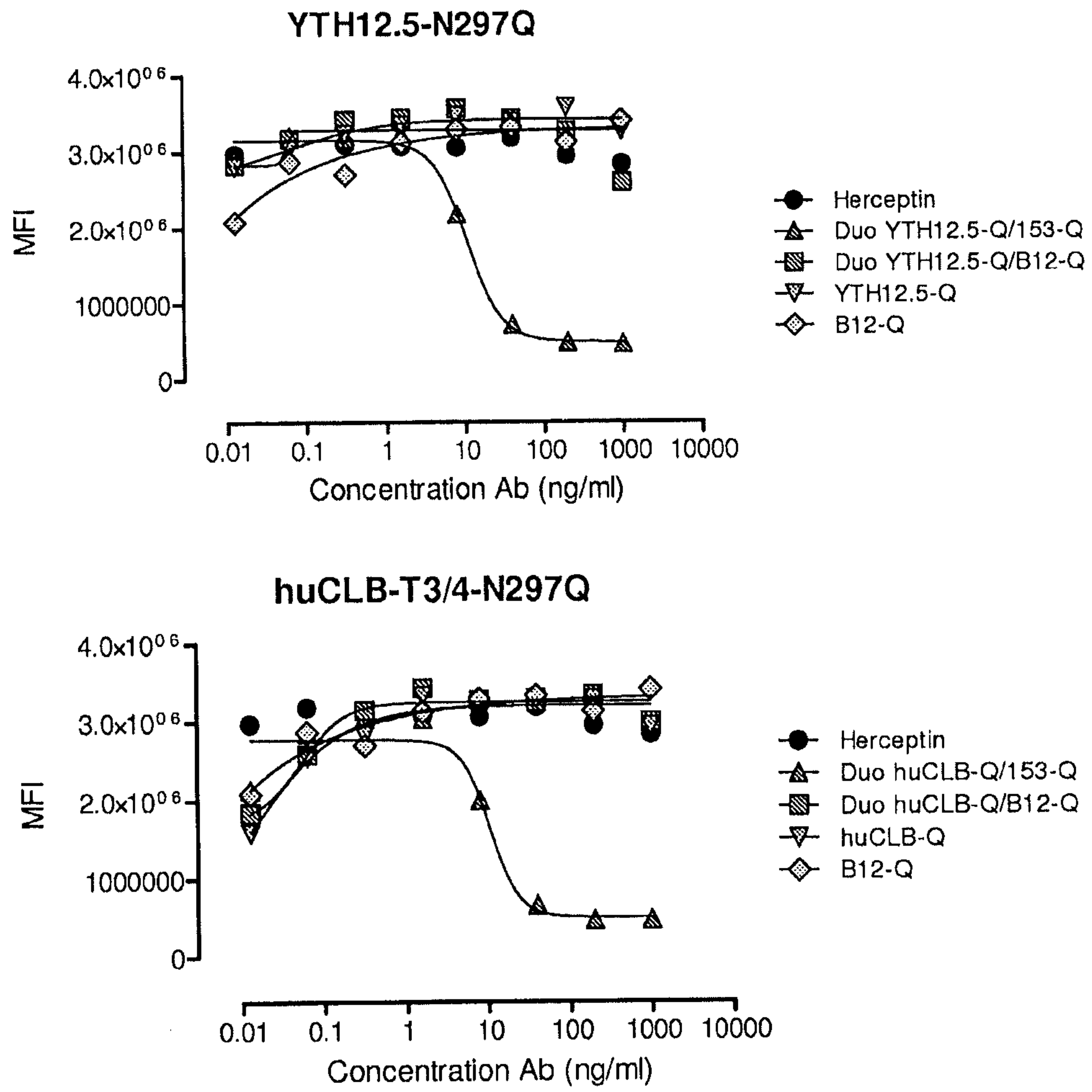


Figure 14

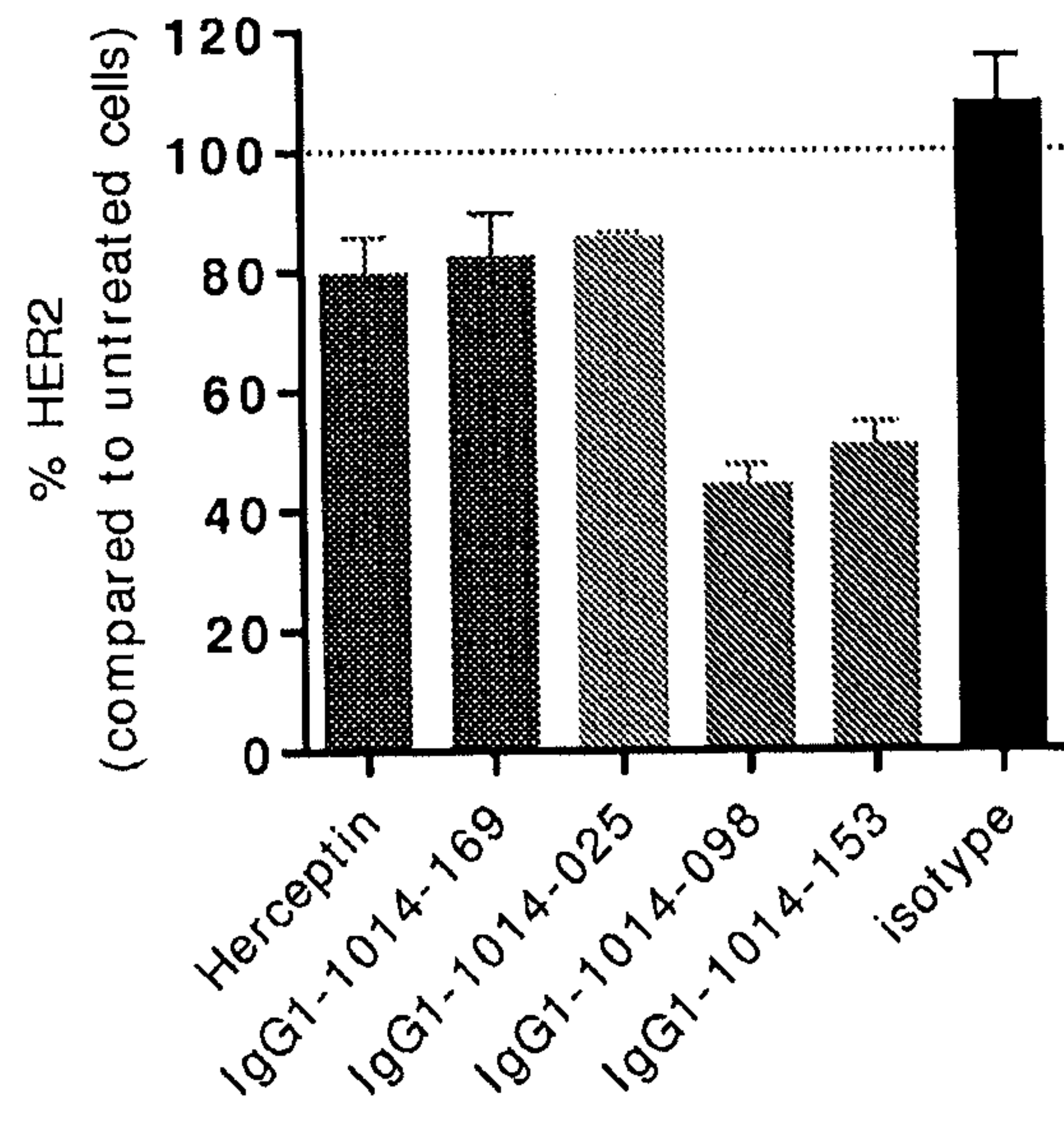


Figure 15

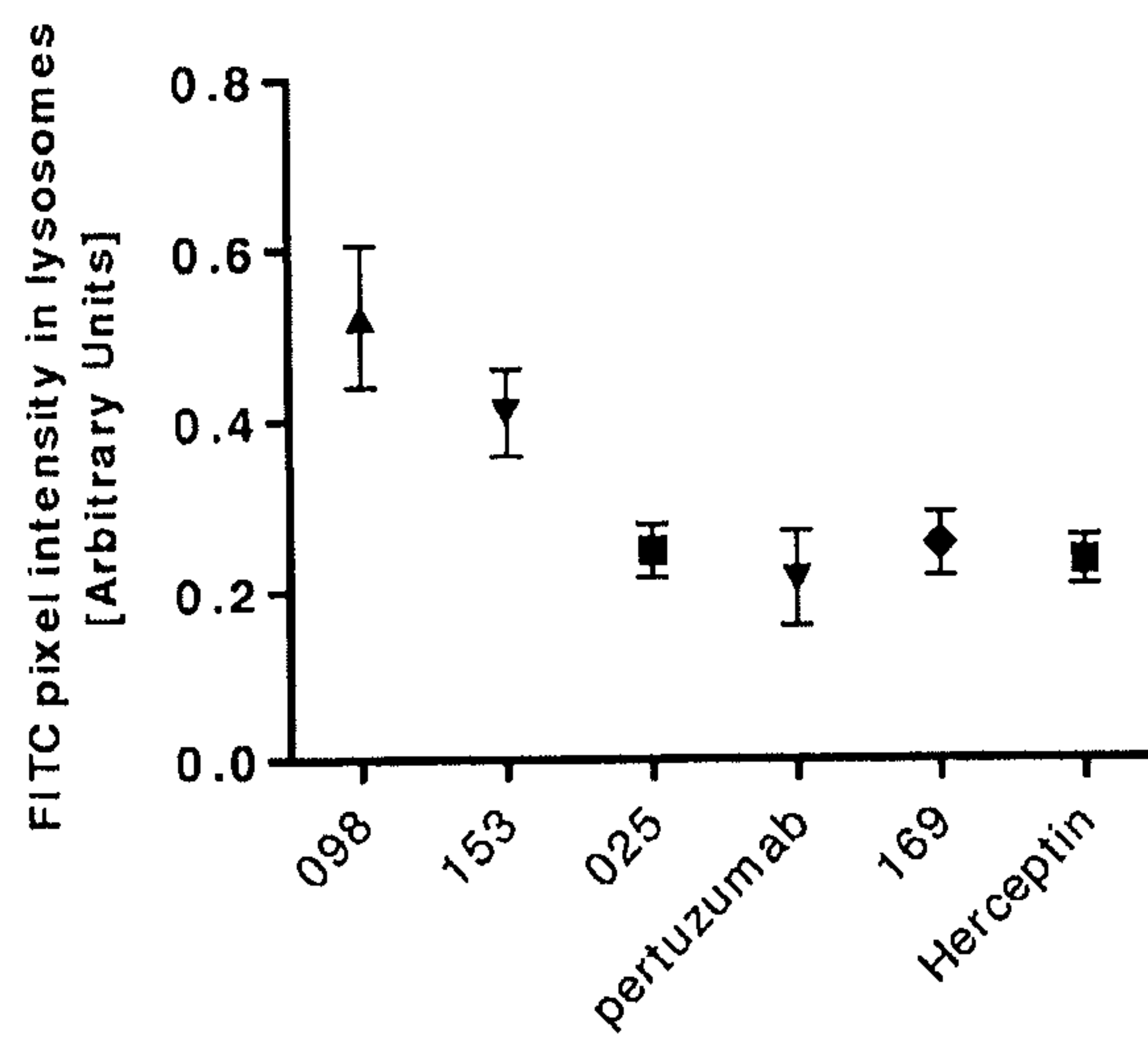


Figure 16

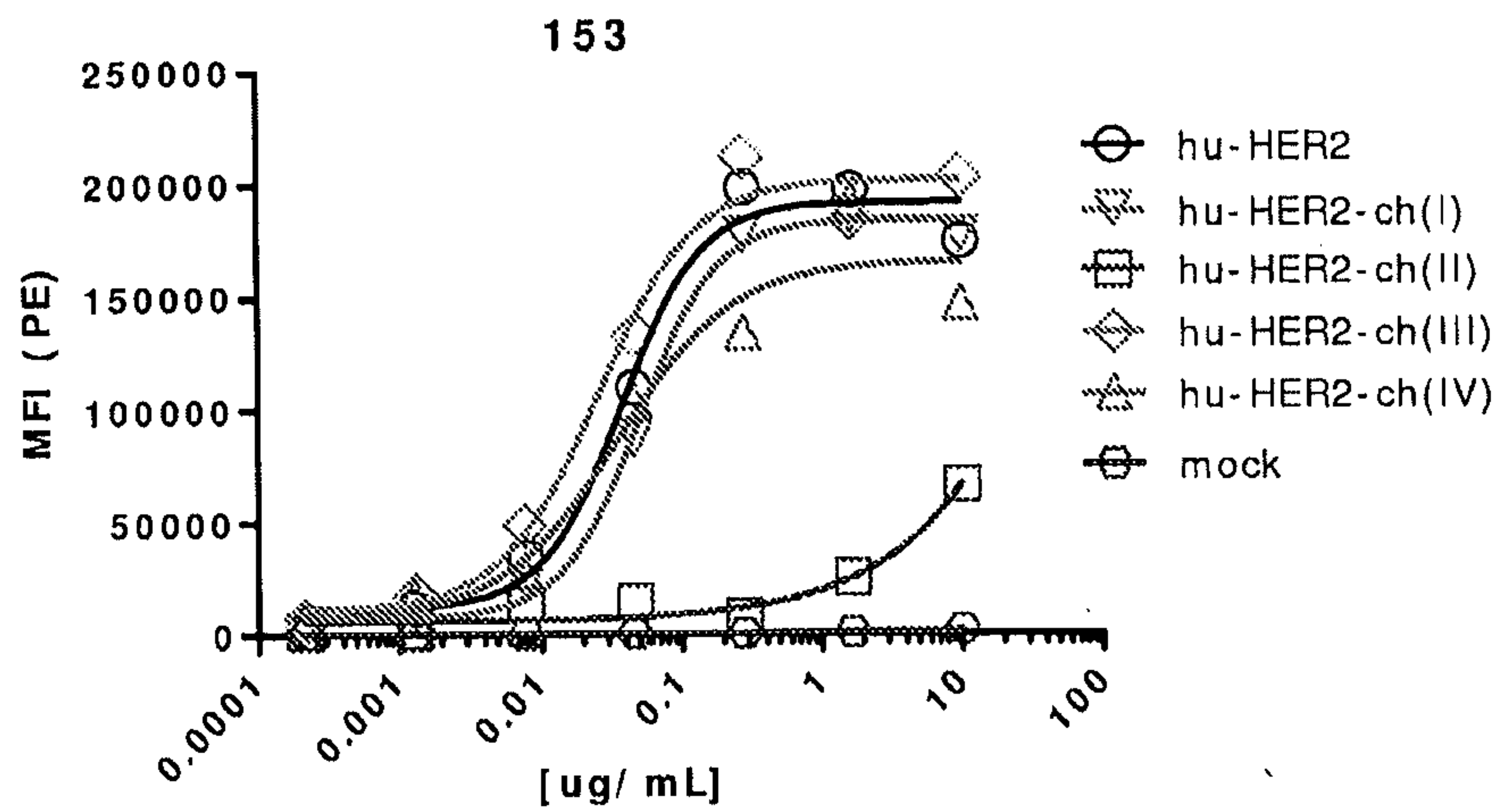


Figure 17A

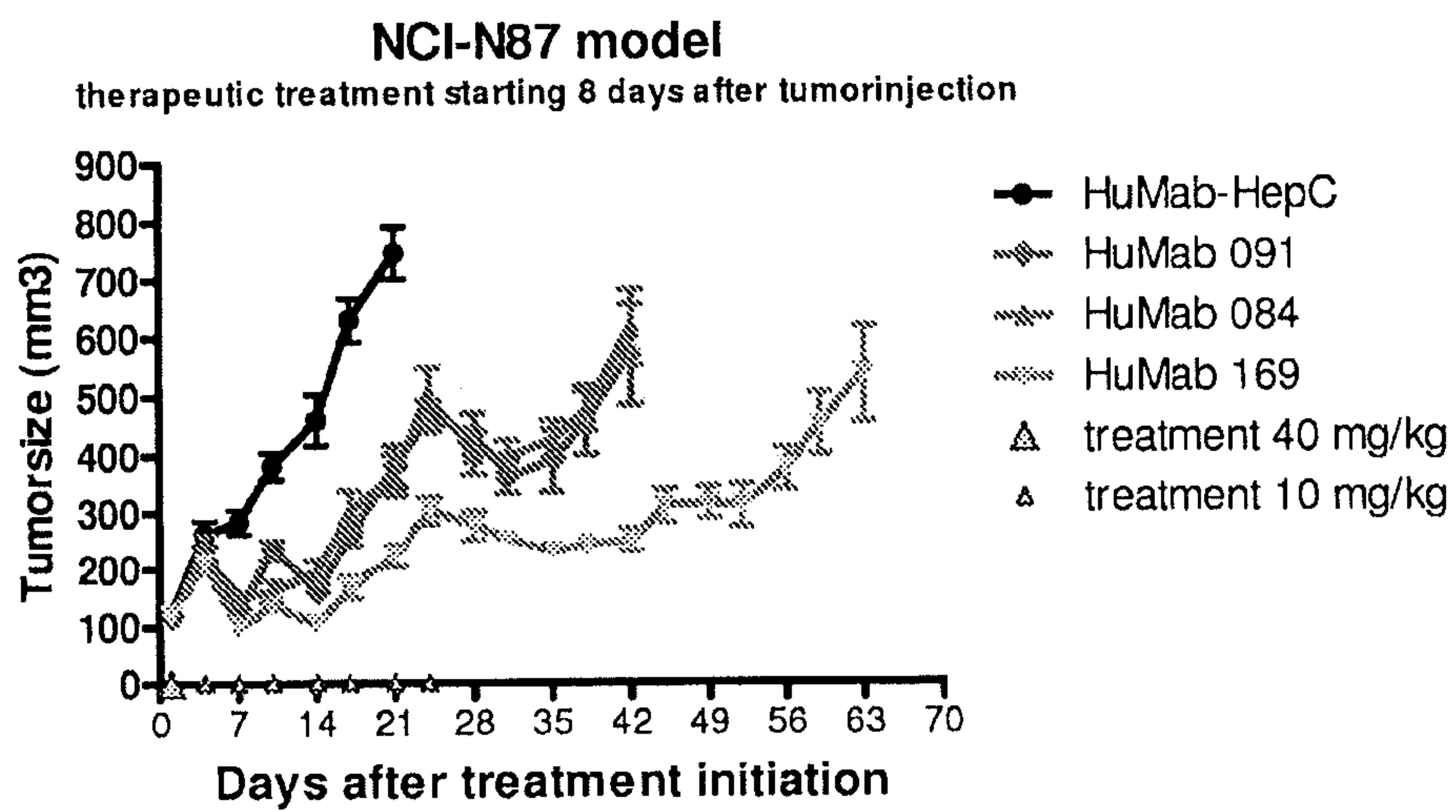


Figure 17B

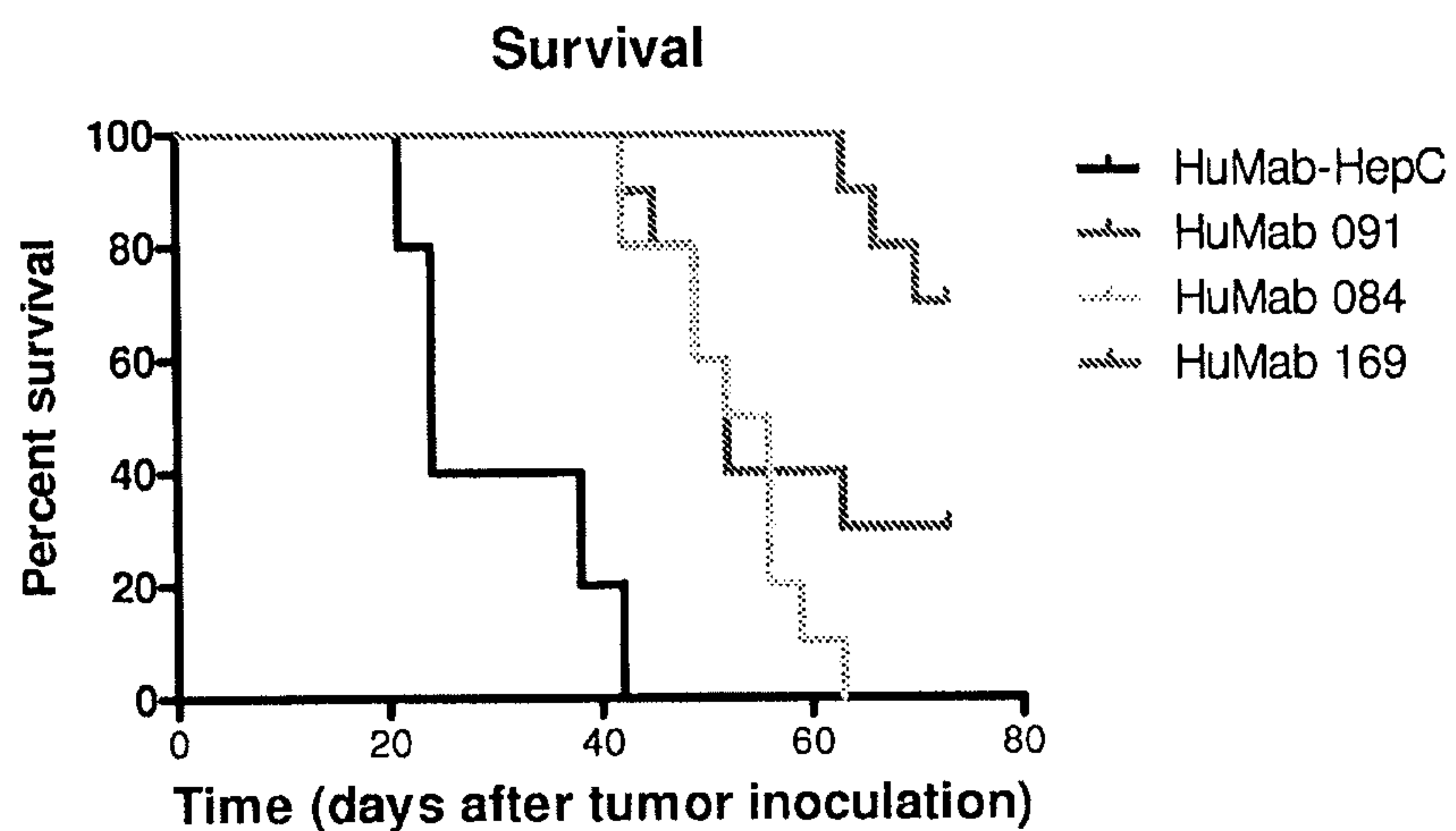


Figure 18A

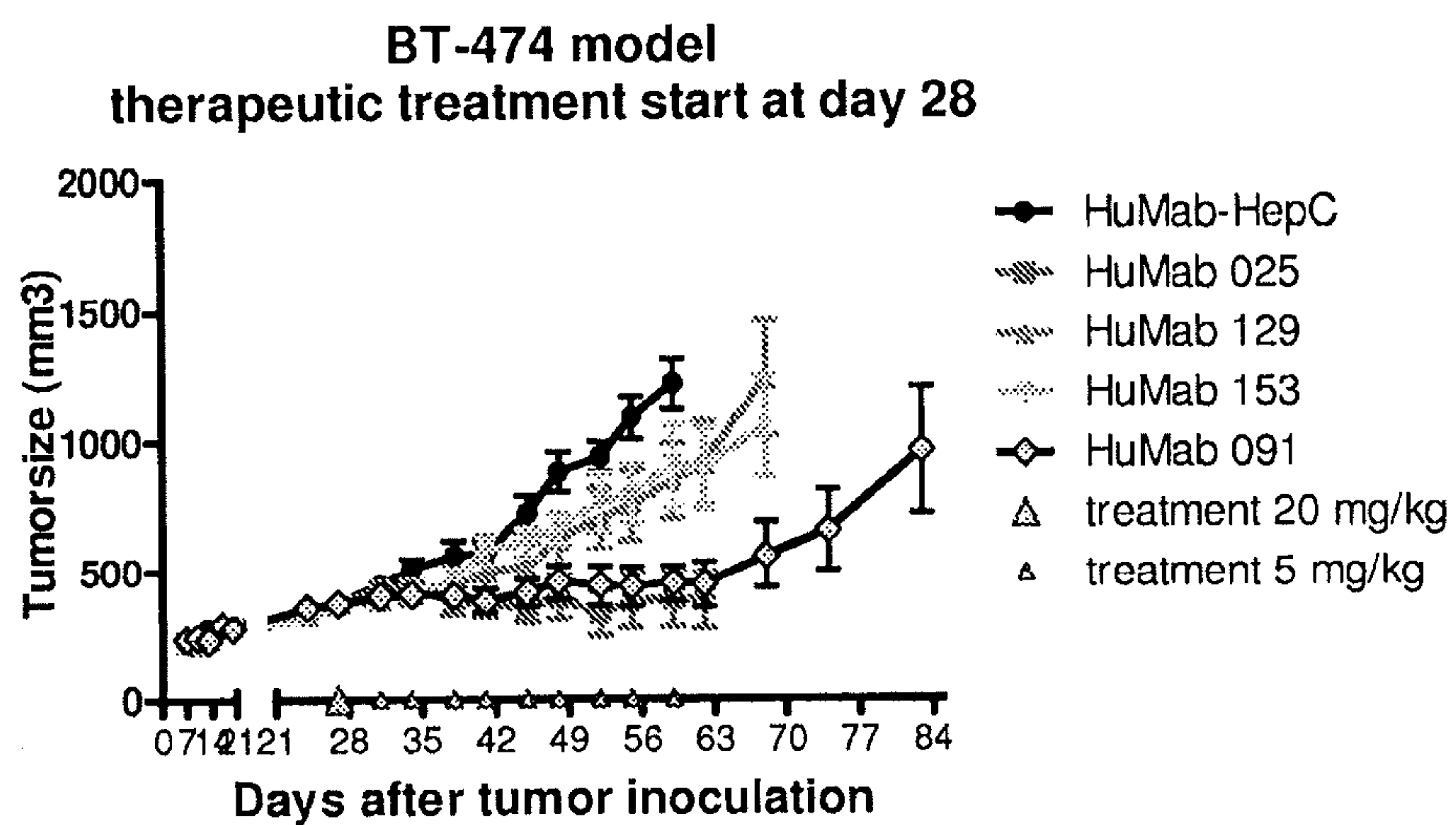
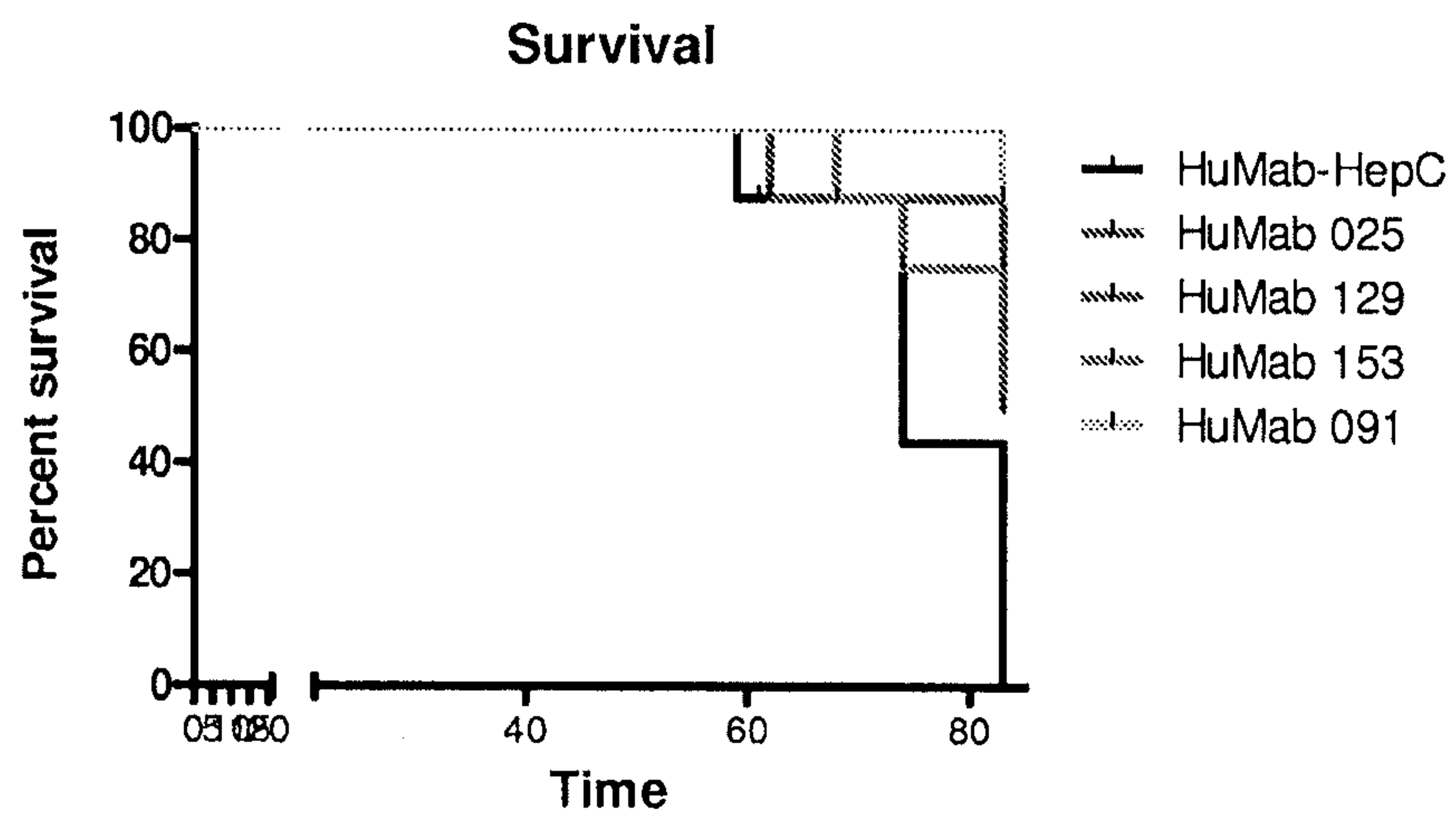


Figure 18B



# IgHV4-34-01 / IGHJ4-02 – VH alignment (Group 2, No. 1)

|                   |                                                                     |
|-------------------|---------------------------------------------------------------------|
| IgHV4-34-01       | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWSWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR  |
| <b>TH1014-025</b> | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLKSR |
| VH1014-001        | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWNWIRQPPGKGLEWIGEINHSGSTNYNPSLKSR  |
| VH1014-143        | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSGYYWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLMSR |
| VH1014-019        | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLKSR |
| VH1014-021        | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYYWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLKSR |
| VH1014-027        | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSDYFWNWIRQPPGKGLEWIGEIHHSNSTNYPNPSLKSR |
| Consensus         | QVQLQQWGAGLLKPSETLSLTCAVYGGSFSSXXWIRQPPGKGLEWIGEIXHXGSXNYPNPSLXSR   |

|                   |                                                         |
|-------------------|---------------------------------------------------------|
| IgHV4-34-01       | VTISVDTSKNQFSLKLSSVTAADTAVYYCAR-----YFDYWGQGTLVTVSS     |
| <b>TH1014-025</b> | VTISVDTSKNQFSLKLSSVTAADTAVYYCARGYYDSGVYYFDYWAQGTLVTVSS  |
| VH1014-001        | VTISVDTSKNQFSLKLSSVTAADTAVYYCARGNYGSGYYFDLWGRGTQVTVSS   |
| VH1014-143        | VTISVDTSKNQFSLQLSSVTAADTAVYYCARGYYGSGYYFDYWGQGTLVTVSS   |
| VH1014-019        | VTISVDTSKQFSLKLSSVTAADTAVYYCARGYYDSGVYYFDYWAQGTLVTVSS   |
| VH1014-021        | VTISVDTSKNQFSLKLSSVTAADTAVYYCARGYYASGVYYFDYWGQGTLVTVSS  |
| VH1014-027        | VTISVDTSKNQFSLNLSSVTAADTAVYYCARGLICSGYYFDYWDQGTGLVTVSS  |
| Consensus         | VTISVDTSKXQFSLXLSSVTAADTAVYYCARGXXXSGXYYFDXWXXGTXTVTVSS |