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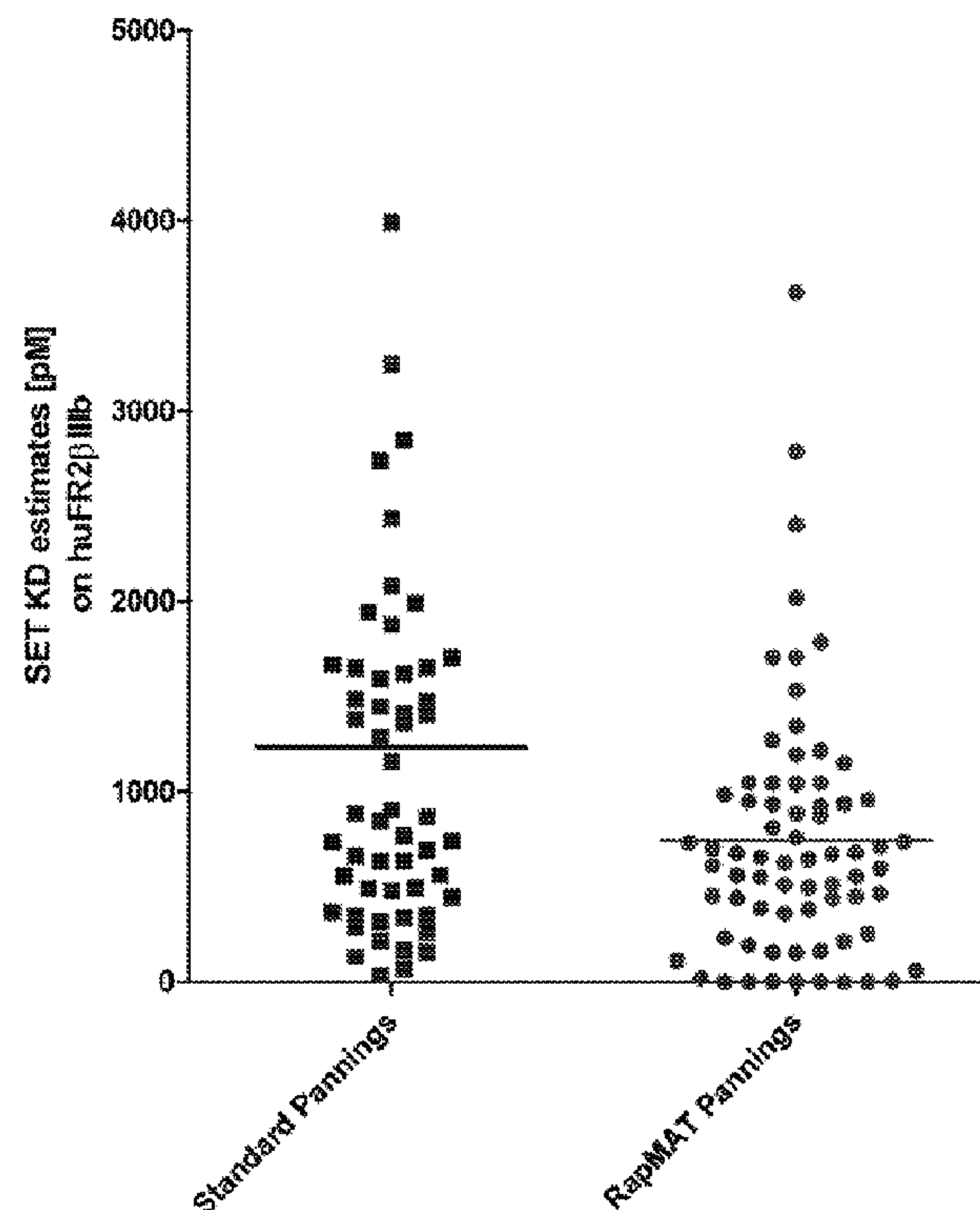
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FIG. 1



(57) **Abrégé/Abstract:**

The present invention relates to anti-FGFR2 and FGFR4 antibodies, antibody fragments, antibody drug conjugates, and their uses for the treatment of cancer.

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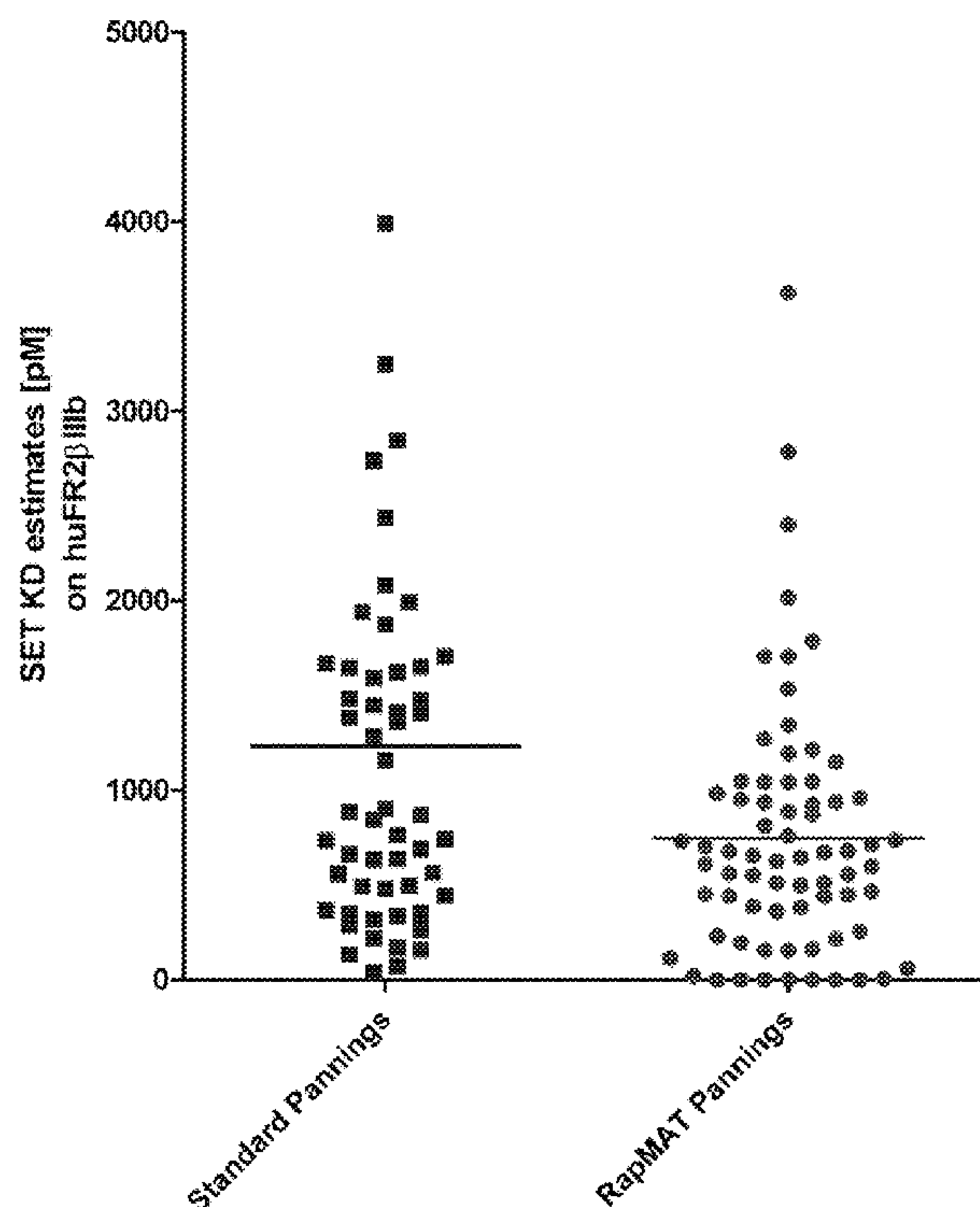
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(54) Title: ANTIBODY DRUG CONJUGATES AND CORRESPONDING ANTIBODIES

FIG. 1



(57) Abstract: The present invention relates to anti-FGFR2 and FGFR4 antibodies, antibody fragments, antibody drug conjugates, and their uses for the treatment of cancer.

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ANTIBODY DRUG CONJUGATES

FIELD OF THE INVENTION

[0001] The present invention generally relates to anti-FGFR2 and FGFR4 antibodies, antibody fragments, antibody drug conjugates, and their uses for the treatment of cancer.

BACKGROUND OF THE INVENTION

Fibroblast Growth Factor Receptors

[0002] Fibroblast growth factors (FGFs) that signal through FGF receptors (FGFRs) regulate fundamental developmental pathways and are expressed in a wide variety of tissues. FGFs stimulate proliferation, cell migration, differentiation, and play a major role in skeletal and limb development, wound healing, tissue repair, hematopoiesis, angiogenesis, and tumorigenesis (see, *e.g.*, Turner and Crose, *Nature Reviews Cancer* 10: 116 – 129 (2010)).

[0003] The mammalian FGF family comprises many ligands, which exert their actions through four highly conserved transmembrane tyrosine kinase receptors, FGFR1, FGFR2, FGFR3, and FGFR4. A typical FGFR has a signal peptide that is cleaved off, three immunoglobulin (Ig)-like domains (Ig domain I, II and III), an acidic box, a transmembrane domain, and a split tyrosine kinase domain (see, *e.g.*, Ullrich and Schlessinger, *Cell* 61: 203 (1990); Johnson and Williams, *Adv. Cancer Res.* 60: 1-41 (1992)).

[0004] Additionally, alternative splicing of the transcribed receptor genes results in a variety of receptor isoforms, including soluble, secreted FGFRs, FGFRs with truncated COOH-terminal domain, FGFRs with either two or three Ig-like domains, as well as FGFR isoforms arising via alternative splicing of the third Ig-like domain occurs only for FGFR1, FGFR2, and FGFR3 and specifies the second half of the third Ig-like domain, resulting in either the IIIb or the IIIc isoform of the receptor. The 2nd and the 3rd Ig-like domains of the receptors are necessary and sufficient for ligand binding, whereas the first Ig-like domain is thought to play a role in receptor autoinhibition. Thus, the different receptors and their isoforms display different ligand-binding specificities (see, *e.g.*, Haugsten *et al.*, *Mol. Cancer Res.* 8:1439-1452 (2010)).

[0005] The FGFs can also bind to heparin sulfate proteoglycans (HSPG), besides binding to distinct FGFRs and their splice variants. Thereby a dimeric FGF-FGFR-HSPG ternary

complex forms on the cell surface. The ternary complex is stabilized by multiple interactions between the different components in the complex. Two FGF-binding sites, a heparin-binding site, and a receptor-receptor interaction site have been identified within the Ig-like domains II and III of the receptor. (Haugsten *et al.*, 2010).

[0006] Binding of FGFs to FGFRs induces receptor dimerization, which enables transphosphorylation of a tyrosine in the activation loop of the kinase domain. The active FGFRs have been shown to phosphorylate multiple intracellular proteins such as FRS2, and PLC γ (Eswarakumar *et al.*, Cytokine Growth Factor Rev 16:139-149 (2005)). FGFR signaling produces distinct biological responses in different cell types, ranging from stimulation of cell proliferation and survival to growth arrest, migration, and differentiation.

FGFRs and Cancer

[0007] FGF signaling mediates a powerful combination of effects: self-sufficiency in growth/survival, neoangiogenesis and tumor cell migration. Consequently, FGF signaling has the potential of being strongly oncogenic once the tight regulation exerted on its physiological functions is lost (see, *e.g.*, Heinzle *et al.*, Expert Opin. Ther. Targets 15(7):829-846 (2011)).

[0008] Gene amplification and /or overexpression of FGFR1, FGFR2 and FGFR4 has been implicated in breast cancer (Penault-Llorca *et al.*, Int J Cancer 1995; Theillet *et al.*, Genes Chrom. Cancer 1993; Adnane *et al.*, Oncogene 1991; Jaakola *et al.*, Int J Cancer 1993; Yamada *et al.*, Neuro Res 2002). Overexpression of FGFR1 and FGFR4 is also associated with pancreatic adenocarcinomas and astrocytomas (Kobrin *et al.*, Cancer Research 1993; Yamanaka *et al.*, Cancer Research 1993; Shah *et al.*, Oncogene 2002; Yamaguchi *et al.*, PNAS 1994; Yamada *et al.*, Neuro Res 2002). Prostate cancer has also been related to FGFR1 overexpression (Giri *et al.*, Clin Cancer Res 1999). FGFR4 overexpression has been implicated in rhabdomyosarcomas (Khan *et al.*, Nature Medicine 2001, Baird *et al.*, Cancer Res 2005). A subset of alveolar rhabdomyosarcomas harbor the PAX3-FOXO translocation yielding novel fusion protein that directly transcriptionally activate FGFR4 and result in increased FGFR4 protein expression (Cao *et al.*, Cancer Res 2010; Davicioni *et al.*, Cancer Res 2006).

[0009] In general FGFR2 and FGFR1 are more commonly deregulated by gene amplification. In gastric cancer, an amplified FGFR2 gene is associated with poor prognosis (Kunii *et al.*, Cancer Res. 68:2340-2348 (2008)). A FGFR2-IIIb to IIIc switch can also be a sign

of tumor progression, epithelial-mesenchymal transition and high invasiveness in bladder and prostate cancers. Switching in this case substitutes a IIIb receptor variant that exerts anti-tumorigenic activity with a protumorigenic IIIc receptor (see, *e.g.*, Heinzle *et al.*, 2011). So far FGFR2 mutations have been implicated in skin, endometrial, ovary, and lung cancer. FGFR4 mutations have been implicated in rhabdomyosarcoma, lung, and breast cancer (see, *e.g.*, Heinzle *et al.*, 2011).

Antibody drug conjugates

[0010] Antibody drug conjugates (“ADCs”) have been used for the local delivery of cytotoxic agents in the treatment of cancer (see *e.g.*, Lambert, Curr. Opinion In Pharmacology 5:543-549, 2005). ADCs allow targeted delivery of the drug moiety where maximum efficacy with minimal toxicity may be achieved. As more ADCs show promising clinical results, there is an increased need to develop new therapeutics for cancer therapy.

SUMMARY OF THE INVENTION

[0011] The present invention provides antibody drug conjugates of the formula Ab—(L—(D)_m)_n, or a pharmaceutically acceptable salt thereof, wherein Ab is an antibody or antigen binding fragment thereof that specifically binds to both human FGFR2 and FGFR4; L is a linker; D is a drug moiety; m is an integer from 1 to 8; and n is an integer from 1 to 10. In some embodiments, m is 1. In some embodiments, n is 3 or 4. In a specific embodiment, m is 1 and n is 3 or 4.

[0012] The present invention provides antibodies or antigen binding fragments thereof that specifically bind to human FGFR2 and human FGFR4. In some embodiments, the antibodies or antigen binding fragments described herein specifically bind to human FGFR4 and all isoforms of human FGFR2. In some embodiment, the antibodies or antigen binding fragments described herein specifically bind to an epitope on human FGFR2 comprising amino acid residues 176 (Lys) and 210 (Arg) of SEQ ID NO:137. In some embodiments, the antibodies or antigen binding fragments described herein specifically bind amino acid residues 173 (Asn), 174 (Thr), 175 (Val), 176 (Lys), 178 (Arg), 208 (Lys), 209 (Val), 210 (Arg), 212 (Gln), 213 (His), 217 (Ile), and 219 (Glu) of SEQ ID NO:137. The present invention further provides antibody drug conjugates comprising the antibodies or antigen binding fragments described herein.

[0013] In some embodiments, the antibodies or antigen binding fragments described herein specifically bind to an epitope of human FGFR2 comprising or consisting SEQ ID NO:136 or SEQ ID NO:141. The present invention further provides antibody drug conjugates comprising the antibodies or antigen binding fragments described herein.

[0014] The antibody, antigen binding fragments, and the antibody drug conjugates of the present invention also specifically bind to human FGFR4. In some embodiments, they specifically bind to D1 and D2 domains of human FGFR4. In some embodiments, they specifically bind to D1 or D2 domain of human FGFR4. In some embodiments, the antibodies or antigen binding fragments described herein specifically bind to an epitope on human FGFR4 comprising amino acid residues 169 (Lys) and 203 (Arg) of SEQ ID NO:142. In some embodiments, the antibodies or antigen binding fragments described herein specifically bind amino acid residues 150 (Thr), 151 (His), 154 (Arg), 157 (Lys), 160 (His), 166 (Asn), 167 (Thr), 168 (Val), 169 (Lys), 171 (Arg), 173 (Pro), 174 (Ala), 201 (Arg), 202 (Leu), 203 (Arg), 204 (His), 205 (Gln), 206 (His), 207 (Trp), 210 (Val) and 212 (Glu) of SEQ ID NO:142. In some embodiments, the antibodies or antigen binding fragments described herein specifically bind to an epitope of human FGFR4 comprising or consisting SEQ ID NO:132 or SEQ ID NO:133. The present invention further provides antibody drug conjugates comprising the antibodies or antigen binding fragments described herein.

[0015] In some embodiments, the antibodies or antigen binding fragments described herein specifically bind to an epitope of human FGFR2 comprising amino acid residues 176 (Lys) and 210 (Arg) of SEQ ID NO:137 and an epitope on human FGFR4 comprising amino acid residues 169 (Lys) and 203 (Arg) of SEQ ID NO:142.

[0016] In some embodiments, the antibodies or antigen binding fragments described herein specifically bind to an epitope comprising amino acids 160-189 of SEQ ID NO: 137 and/or amino acids 198-216 of SEQ ID NO: 137. In some embodiments, the antibodies or antigen binding fragments described herein specifically bind to an epitope comprising amino acids 150-174 of SEQ ID NO: 142 and/or amino acids 201-212 of SEQ ID NO: 142. In some embodiments, the antibodies or antigen binding fragments described herein specifically bind to an epitope comprising amino acids 160-189 of SEQ ID NO: 137 and/or amino acids 198-216 of SEQ ID NO: 137 and a second epitope comprising amino acids 150-174 of SEQ ID NO: 142 and/or amino acids 201-212 of SEQ ID NO: 142.

[0017] In some embodiments, the present invention provides antibodies described in Table 1, antigen binding fragments thereof, and antibody drug conjugates comprising such antibodies or antigen binding fragments. In some embodiments, the present invention provides antibodies and antigen binding fragments comprising a heavy chain variable region that comprises: (a) a VH CDR1 of SEQ ID NO: 1, (b) a VH CDR2 of SEQ ID NO: 2, and (c) a VH CDR3 of SEQ ID NO: 3, wherein the CDR is defined in accordance with the Kabat definition. In some embodiments, the antibodies or antigen binding fragments of the invention comprise a heavy chain variable region that comprises: (a) a VH CDR1 of SEQ ID NO: 1, (b) a VH CDR2 of SEQ ID NO: 2, and (c) a VH CDR3 of SEQ ID NO: 3; and a light chain variable region that comprises: (a) a VL CDR1 of SEQ ID NO: 11, (b) a VL CDR2 of SEQ ID NO: 12, and (c) a VL CDR3 of SEQ ID NO: 13, wherein the CDR is defined in accordance with the Kabat definition. The present invention further provides antibody drug conjugates that comprise such antibodies or antigen binding fragments.

[0018] In some embodiments, the antibodies or antigen binding fragments described herein comprise a VH region of SEQ ID NO: 7 and a VL region of SEQ ID NO: 17. In some embodiments, the antibodies described herein consist of a heavy chain of SEQ ID NO: 9 and a light chain of SEQ ID NO: 19. The present invention also provides antibody drug conjugates comprising such antibodies or antigen binding fragments thereof.

[0019] In some embodiments, the antibodies or antigen binding fragments described herein are antibodies or antigen fragments that cross compete with an antibody consists of a heavy chain of SEQ ID NO: 9 and a light chain of SEQ ID NO: 19 for binding to human FGFR2 and FGFR4. The present invention also provides antibody drug conjugates comprising such antibodies or antigen binding fragments.

[0020] In some embodiments, the antibodies or antigen binding fragments described herein have enhanced ADCC activity as compared to an antibody consisting of a heavy chain of SEQ ID NO: 9 and a light chain of SEQ ID NO: 19. In some embodiments, the antibodies or antigen binding fragments described herein do not have enhanced ADCC activity as compared to an antibody consisting of a heavy chain of SEQ ID NO: 9 and a light chain of SEQ ID NO: 19. The present invention also provides antibody drug conjugates comprising such antibodies or antigen binding fragments.

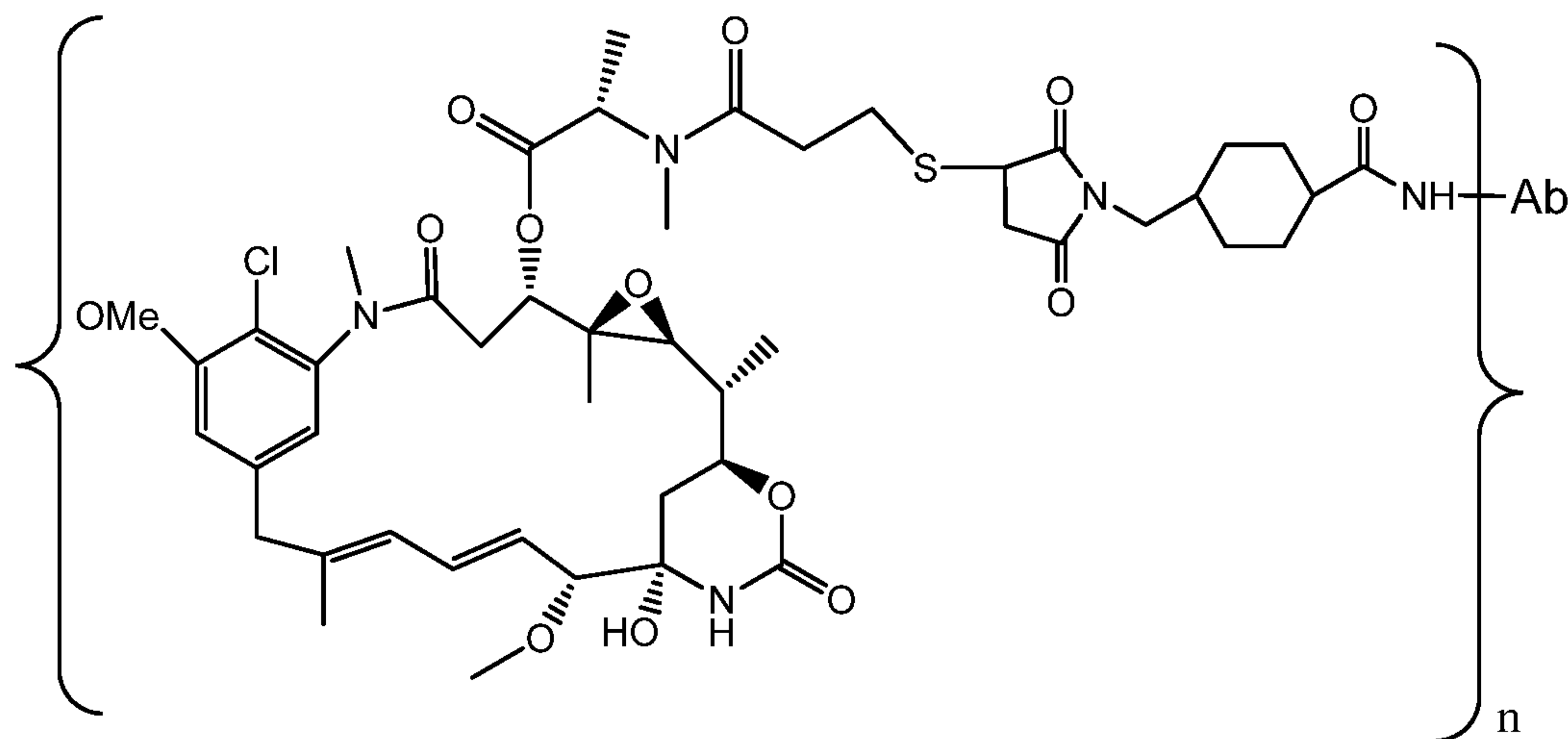
[0021] In some embodiments, the antibodies described herein are human or humanized antibodies. In some embodiments, the antibodies described herein are monoclonal antibodies. In some embodiments, the antibodies described herein are monoclonal human or humanized antibodies.

[0022] In some embodiments, the present invention provides antibody drug conjugates comprising an antibody or antigen binding fragment described herein, and a drug moiety, wherein the drug moiety is linked to the antibody or antigen binding fragment via a linker, and wherein said linker is selected from the group consisting of a cleavable linker, a non-cleavable linker, a hydrophilic linker, a procharged linker and a dicarboxylic acid based linker. In some embodiments, the linker is derived from a cross-linking reagent selected from the group consisting of: N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC) or 2,5-dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1). In a specific embodiment, the linker is derived from the cross-linking reagent N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC).

[0023] In some embodiments, the present invention provides antibody drug conjugates comprising an antibody or antigen binding fragment described herein, and a drug moiety, wherein said drug moiety is selected from a group consisting of: a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor. In a specific embodiment, the drug moiety of an antibody drug conjugate of the invention is maytansinoid. In another specific embodiment, the drug moiety of an antibody drug conjugate of the invention is

N(2')- deacetyl-N(2')-(3-mercapto-1-oxopropyl)-maytansine (DM1) or N(2')-deacetyl-N2-(4-mercapto-4-methyl- 1 -oxopentyl)-maytansine (DM4).

[0024] In one embodiment, the present invention provides antibody drug conjugates having the following formula:



wherein Ab is an antibody or antigen binding fragment thereof comprising a heavy chain CDR1 of SEQ ID NO: 1, a heavy chain CDR2 of SEQ ID NO: 2, a heavy chain CDR3 of SEQ ID NO: 3, and a light chain CDR1 of SEQ ID NO: 11, a light chain CDR2 of SEQ ID NO: 12, a light chain CDR3 of SEQ ID NO: 13, wherein the CDR is defined in accordance with the Kabat definition; and n is 1 to 10; or a pharmaceutically acceptable salt thereof. In a specific embodiment, n is 3 or 4. In one embodiment, Ab is an antibody comprising a VH region of SEQ ID NO: 7 and a VL region of SEQ ID NO: 17. In another embodiment, Ab is an antibody consist of a heavy chain of SEQ ID NO: 9 and a light chain of SEQ ID NO: 19.

[0023] The present invention also provides pharmaceutical compositions comprising the antibody drug conjugate described herein and a pharmaceutically acceptable carrier. In some embodiments, the pharmaceutical compositions of the invention is prepared as a lyophilisate. In some embodiments, the lyophilisate comprises an antibody drug conjugate described herein, sodium succinate, and polysorbate 20.

[0024] The present invention provides methods of treating an FGFR2 positive or FGFR4 positive cancer in a patient in need thereof, comprising administering to said patient an antibody drug conjugate or a pharmaceutical composition described herein. In some embodiments, the

cancer is selected from the group consisting of gastric cancer, breast cancer, rhabdomyosarcoma, liver cancer, adrenal cancer, lung cancer, esophageal cancer, colon cancer and endometrial cancer. In certain embodiments, the cancer is selected from alveolar rhabdomyosarcoma and embryonal rhabdomyosarcoma. In some embodiments, the treatment methods described herein further comprise administering to said patient a tyrosine kinase inhibitor, an IAP inhibitor, a Bcl2 inhibitor, an MCL1 inhibitor, or another FGFR2 inhibitor. In a specific embodiment, the treatment method comprising administering to a patient in need thereof an antibody drug conjugate described herein in combination with 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea or a pharmaceutically acceptable salt thereof, for example the phosphate salt thereof. In certain aspects the invention also provides methods of treating cancer resistant to other tyrosine kinase inhibitors, including but not limited to, EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGFR inhibitors and Met inhibitors.

[0025] The present invention further provides antibody drug conjugates described herein for use as a medicament. The present invention provides antibody drug conjugates or pharmaceutical compositions for use in the treatment of an FGFR2 positive or FGFR4 positive cancer. The present invention provides antibody drug conjugates or pharmaceutical compositions for use in the treatment of resistant cancer, for example, cancer resistant to other tyrosine kinase inhibitors, including but not limited to, EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGFR inhibitors and Met inhibitors.

[0026] In some embodiments, the antibodies or antigen binding fragments described herein are a single chain antibody (scFv).

[0027] The present invention also provides nucleic acids that encodes the antibodies or antigen binding fragments described herein. In some embodiments, the present invention provides nucleic acids that encodes the antibodies or antigen binding fragments as those described in Table 1. The present invention further provides vectors comprising the nucleic acid described herein, and host cells comprising such vectors.

[0028] The present invention provides processes for producing an antibody or antigen binding fragment comprising cultivating the host cells described herein and recovering the antibody from the culture.

[0029] In one embodiment, the present invention provides a process for producing an anti-FGFR2 and FGFR4 antibody drug conjugate comprising: (a) chemically linking SMCC to a drug moiety DM-1; (b) conjugating said linker-drug to the antibody recovered from the cell culture; and (c) purifying the antibody drug conjugate. In some embodiments, the antibody drug conjugates made according the process have an average DAR, measured with a UV spectrophotometer, about 3.5.

[0030] The present invention also provides diagnostic reagents comprising an antibody or antigen binding fragment described herein, or the antibody drug conjugate described herein which is labeled. In some embodiments, the label is selected from the group consisting of a radiolabel, a fluorophore, a chromophore, an imaging agent, and a metal ion.

Definitions

[0031] Unless stated otherwise, the following terms and phrases as used herein are intended to have the following meanings:

[0032] The term “alkyl” refers to a monovalent saturated hydrocarbon chain having the specified number of carbon atoms. For example, C₁₋₆ alkyl refers to an alkyl group having from 1 to 6 carbon atoms. Alkyl groups may be straight or branched. Representative branched alkyl groups have one, two, or three branches. Examples of alkyl groups include, but are not limited to, methyl, ethyl, propyl (n-propyl and isopropyl), butyl (n-butyl, isobutyl, sec-butyl, and t-butyl), pentyl (n-pentyl, isopentyl, and neopentyl), and hexyl.

[0033] The term “antibody” as used herein refers to a polypeptide of the immunoglobulin family that is capable of binding a corresponding antigen non-covalently, reversibly, and in a specific manner. For example, a naturally occurring IgG antibody is a tetramer comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH1, CH2 and CH3. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The light chain constant region is comprised of one domain, CL. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1,

FR2, CDR2, FR3, CDR3, and FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (C1q) of the classical complement system.

[0034] The term “antibody” includes, but is not limited to, monoclonal antibodies, human antibodies, humanized antibodies, chimeric antibodies, and anti-idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to antibodies of the invention). The antibodies can be of any isotype/class (*e.g.*, IgG, IgE, IgM, IgD, IgA and IgY), or subclass (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2).

[0035] “Complementarity-determining domains” or “complementary-determining regions (“CDRs”) interchangeably refer to the hypervariable regions of VL and VH. The CDRs are the target protein-binding site of the antibody chains that harbors specificity for such target protein. There are three CDRs (CDR1-3, numbered sequentially from the N-terminus) in each human VL or VH, constituting about 15-20% of the variable domains. The CDRs are structurally complementary to the epitope of the target protein and are thus directly responsible for the binding specificity. The remaining stretches of the VL or VH, the so-called framework regions, exhibit less variation in amino acid sequence (Kuby, Immunology, 4th ed., Chapter 4. W.H. Freeman & Co., New York, 2000).

[0036] The positions of the CDRs and framework regions can be determined using various well known definitions in the art, *e.g.*, Kabat, Chothia, international ImMunoGeneTics database (IMGT) (on the worldwide web at imgt.cines.fr/), and AbM (see, *e.g.*, Johnson *et al.*, Nucleic Acids Res., 29:205-206 (2001); Chothia and Lesk, J. Mol. Biol., 196:901-917 (1987); Chothia *et al.*, Nature, 342:877-883 (1989); Chothia *et al.*, J. Mol. Biol., 227:799-817 (1992); Al-Lazikani *et al.*, J.Mol.Biol., 273:927-748 (1997)). Definitions of antigen combining sites are also described in the following: Ruiz *et al.*, Nucleic Acids Res., 28:219–221 (2000); and Lefranc, M.P., Nucleic Acids Res., 29:207-209 (2001); MacCallum *et al.*, J. Mol. Biol., 262:732-745 (1996); and Martin *et al.*, Proc. Natl. Acad. Sci. USA, 86:9268–9272 (1989); Martin *et al.*, Methods Enzymol., 203:121–153 (1991); and Rees *et al.*, In Sternberg M.J.E. (ed.), Protein Structure Prediction, Oxford University Press, Oxford, 141–172 (1996).

[0037] Both the light and heavy chains are divided into regions of structural and functional homology. The terms “constant” and “variable” are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VL) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CL) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention, the numbering of the constant region domains increases as they become more distal from the antigen binding site or amino-terminus of the antibody. The N-terminus is a variable region and at the C-terminus is a constant region; the CH3 and CL domains actually comprise the carboxy-terminal domains of the heavy and light chain, respectively.

[0038] The term “antigen binding fragment”, as used herein, refers to one or more portions of an antibody that retain the ability to specifically interact with (*e.g.*, by binding, steric hindrance, stabilizing/destabilizing, spatial distribution) an epitope of an antigen. Examples of binding fragments include, but are not limited to, single-chain Fvs (scFv), camelid antibodies, disulfide-linked Fvs (sdFv), Fab fragments, F(ab') fragments, a monovalent fragment consisting of the VL, VH, CL and CH1 domains; a F(ab)₂ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; a Fd fragment consisting of the VH and CH1 domains; a Fv fragment consisting of the VL and VH domains of a single arm of an antibody; a dAb fragment (Ward *et al.*, Nature 341:544-546, 1989), which consists of a VH domain; and an isolated complementarity determining region (CDR), or other epitope-binding fragments of an antibody.

[0039] Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (“scFv”); see, *e.g.*, Bird *et al.*, Science 242:423-426, 1988; and Huston *et al.*, Proc. Natl. Acad. Sci. 85:5879-5883, 1988). Such single chain antibodies are also intended to be encompassed within the term “antigen binding fragment.” These antigen binding fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

[0040] Antigen binding fragments can also be incorporated into single domain antibodies, maxibodies, minibodies, nanobodies, intrabodies, diabodies, triabodies, tetrabodies, v-NAR and bis-scFv (see, *e.g.*, Hollinger and Hudson, *Nature Biotechnology* 23:1126-1136, 2005). Antigen binding fragments can be grafted into scaffolds based on polypeptides such as fibronectin type III (Fn3) (see U.S. Pat. No. 6,703,199, which describes fibronectin polypeptide monobodies).

[0041] Antigen binding fragments can be incorporated into single chain molecules comprising a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions (Zapata *et al.*, *Protein Eng.* 8:1057-1062, 1995; and U.S. Pat. No. 5,641,870).

[0042] The term “monoclonal antibody” or “monoclonal antibody composition” as used herein refers to polypeptides, including antibodies and antigen binding fragments that have substantially identical amino acid sequence or are derived from the same genetic source. This term also includes preparations of antibody molecules of single molecular composition. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope.

[0043] The term “human antibody”, as used herein, includes antibodies having variable regions in which both the framework and CDR regions are derived from sequences of human origin. Furthermore, if the antibody contains a constant region, the constant region also is derived from such human sequences, *e.g.*, human germline sequences, or mutated versions of human germline sequences or antibody containing consensus framework sequences derived from human framework sequences analysis, for example, as described in Knappik *et al.*, *J. Mol. Biol.* 296:57-86, 2000).

[0044] The human antibodies of the invention may include amino acid residues not encoded by human sequences (*e.g.*, mutations introduced by random or site-specific mutagenesis in vitro or by somatic mutation in vivo, or a conservative substitution to promote stability or manufacturing).

[0045] The term “affinity” as used herein refers to the strength of interaction between antibody and antigen at single antigenic sites. Within each antigenic site, the variable region of the antibody “arm” interacts through weak non-covalent forces with antigen at numerous sites; the more interactions, the stronger the affinity.

[0046] The term “isolated antibody” refers to an antibody that is substantially free of other antibodies having different antigenic specificities. An isolated antibody that specifically binds to one antigen may, however, have cross-reactivity to other antigens. Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals.

[0047] The term “corresponding human germline sequence” refers to the nucleic acid sequence encoding a human variable region amino acid sequence or subsequence that shares the highest determined amino acid sequence identity with a reference variable region amino acid sequence or subsequence in comparison to all other all other known variable region amino acid sequences encoded by human germline immunoglobulin variable region sequences. The corresponding human germline sequence can also refer to the human variable region amino acid sequence or subsequence with the highest amino acid sequence identity with a reference variable region amino acid sequence or subsequence in comparison to all other evaluated variable region amino acid sequences. The corresponding human germline sequence can be framework regions only, complementarity determining regions only, framework and complementarity determining regions, a variable segment (as defined above), or other combinations of sequences or subsequences that comprise a variable region. Sequence identity can be determined using the methods described herein, for example, aligning two sequences using BLAST, ALIGN, or another alignment algorithm known in the art. The corresponding human germline nucleic acid or amino acid sequence can have at least about 90%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the reference variable region nucleic acid or amino acid sequence. Corresponding human germline sequences can be determined, for example, through the publicly available international ImMunoGeneTics database (IMGT) (on the worldwide web at imgt.cines.fr/) and V-base (on the worldwide web at vbase.mrc-cpe.cam.ac.uk).

[0048] The phrase “specifically binds” or “selectively binds,” when used in the context of describing the interaction between an antigen (*e.g.*, a protein) and an antibody, antibody fragment, or antibody-derived binding agent, refers to a binding reaction that is determinative of the presence of the antigen in a heterogeneous population of proteins and other biologics, *e.g.*, in a biological sample, *e.g.*, a blood, serum, plasma or tissue sample. Thus, under certain designated immunoassay conditions, the antibodies or binding agents with a particular binding specificity bind to a particular antigen at least two times the background and do not substantially bind in a significant amount to other antigens present in the sample. In one embodiment, under designated

immunoassay conditions, the antibody or binding agent with a particular binding specificity binds to a particular antigen at least ten (10) times the background and does not substantially bind in a significant amount to other antigens present in the sample. Specific binding to an antibody or binding agent under such conditions may require the antibody or agent to have been selected for its specificity for a particular protein. As desired or appropriate, this selection may be achieved by subtracting out antibodies that cross-react with molecules from other species (*e.g.*, mouse or rat) or other subtypes. Alternatively, in some embodiments, antibodies or antibody fragments are selected that cross-react with certain desired molecules.

[0049] The term “specifically binds” when used in the context of describing the interaction between an epitope and an antibody, antibody fragment, or antibody-derived binding agent, refers to an antibody or antigen binding fragment thereof that finds and interacts (*e.g.*: binds) with its epitope, whether that epitope is linear or conformational. The term “epitope” refers to a site on an antigen to which an antibody or antigen binding fragment of the invention specifically binds. Epitopes can be formed both from contiguous amino acids or noncontiguous amino acids juxtaposed by tertiary folding of a protein. Epitopes formed from contiguous amino acids are typically retained on exposure to denaturing solvents, whereas epitopes formed by tertiary folding are typically lost on treatment with denaturing solvents. An epitope typically includes at least 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14 or 15 amino acids in a unique spatial conformation. Methods of determining spatial conformation of epitopes include techniques in the art, for example, x-ray crystallography and 2-dimensional nuclear magnetic resonance (see, *e.g.*, Epitope Mapping Protocols in Methods in Molecular Biology, Vol. 66, G. E. Morris, Ed. (1996)).

[0050] A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays are routinely used to select antibodies specifically immunoreactive with a protein (see, *e.g.*, Harlow & Lane, Using Antibodies, A Laboratory Manual (1998), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity). Typically a specific or selective binding reaction will produce a signal at least twice over the background signal and more typically at least 10 to 100 times over the background.

[0051] The term “equilibrium dissociation constant (K_D , M)” refers to the dissociation rate constant (k_d , time⁻¹) divided by the association rate constant (k_a , time⁻¹, M⁻¹). Equilibrium dissociation constants can be measured using any known method in the art. The antibodies of the

present invention generally will have an equilibrium dissociation constant of less than about 10^{-7} or 10^{-8} M, for example, less than about 10^{-9} M or 10^{-10} M, in some embodiments, less than about 10^{-11} M, 10^{-12} M or 10^{-13} M.

[0052] The term "bioavailability" refers to the systemic availability (i.e., blood/plasma levels) of a given amount of drug administered to a patient. Bioavailability is an absolute term that indicates measurement of both the time (rate) and total amount (extent) of drug that reaches the general circulation from an administered dosage form.

[0053] As used herein, the phrase "consisting essentially of" refers to the genera or species of active pharmaceutical agents included in a method or composition, as well as any excipients inactive for the intended purpose of the methods or compositions. In some embodiments, the phrase "consisting essentially of" expressly excludes the inclusion of one or more additional active agents other than an antibody drug conjugate of the invention. In some embodiments, the phrase "consisting essentially of" expressly excludes the inclusion of one or more additional active agents other than an antibody drug conjugate of the invention and a second co-administered agent.

[0054] The term "amino acid" refers to naturally occurring, synthetic, and unnatural amino acids, as well as amino acid analogs and amino acid mimetics that function in a manner similar to the naturally occurring amino acids. Naturally occurring amino acids are those encoded by the genetic code, as well as those amino acids that are later modified, *e.g.*, hydroxyproline, γ -carboxyglutamate, and O-phosphoserine. Amino acid analogs refer to compounds that have the same basic chemical structure as a naturally occurring amino acid, i.e., an α -carbon that is bound to a hydrogen, a carboxyl group, an amino group, and an R group, *e.g.*, homoserine, norleucine, methionine sulfoxide, methionine methyl sulfonium. Such analogs have modified R groups (*e.g.*, norleucine) or modified peptide backbones, but retain the same basic chemical structure as a naturally occurring amino acid. Amino acid mimetics refers to chemical compounds that have a structure that is different from the general chemical structure of an amino acid, but that functions in a manner similar to a naturally occurring amino acid.

[0055] The term "conservatively modified variant" applies to both amino acid and nucleic acid sequences. With respect to particular nucleic acid sequences, conservatively modified variants refers to those nucleic acids which encode identical or essentially identical amino acid sequences, or where the nucleic acid does not encode an amino acid sequence, to essentially

identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given protein. For instance, the codons GCA, GCC, GCG and GCU all encode the amino acid alanine. Thus, at every position where an alanine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded polypeptide. Such nucleic acid variations are “silent variations,” which are one species of conservatively modified variations. Every nucleic acid sequence herein which encodes a polypeptide also describes every possible silent variation of the nucleic acid. One of skill will recognize that each codon in a nucleic acid (except AUG, which is ordinarily the only codon for methionine, and TGG, which is ordinarily the only codon for tryptophan) can be modified to yield a functionally identical molecule. Accordingly, each silent variation of a nucleic acid that encodes a polypeptide is implicit in each described sequence.

[0056] For polypeptide sequences, “conservatively modified variants” include individual substitutions, deletions or additions to a polypeptide sequence which result in the substitution of an amino acid with a chemically similar amino acid. Conservative substitution tables providing functionally similar amino acids are well known in the art. Such conservatively modified variants are in addition to and do not exclude polymorphic variants, interspecies homologs, and alleles of the invention. The following eight groups contain amino acids that are conservative substitutions for one another: 1) Alanine (A), Glycine (G); 2) Aspartic acid (D), Glutamic acid (E); 3) Asparagine (N), Glutamine (Q); 4) Arginine (R), Lysine (K); 5) Isoleucine (I), Leucine (L), Methionine (M), Valine (V); 6) Phenylalanine (F), Tyrosine (Y), Tryptophan (W); 7) Serine (S), Threonine (T); and 8) Cysteine (C), Methionine (M) (see, *e.g.*, Creighton, Proteins (1984)). In some embodiments, the term “conservative sequence modifications” are used to refer to amino acid modifications that do not significantly affect or alter the binding characteristics of the antibody containing the amino acid sequence.

[0057] The term “optimized” as used herein refers to a nucleotide sequence that has been altered to encode an amino acid sequence using codons that are preferred in the production cell or organism, generally a eukaryotic cell, for example, a yeast cell, a Pichia cell, a fungal cell, a Trichoderma cell, a Chinese Hamster Ovary cell (CHO) or a human cell. The optimized nucleotide sequence is engineered to retain completely or as much as possible the amino acid sequence originally encoded by the starting nucleotide sequence, which is also known as the “parental” sequence.

[0058] The terms “percent identical” or “percent identity,” in the context of two or more nucleic acids or polypeptide sequences, refers to the extent to which two or more sequences or subsequences that are the same. Two sequences are “identical” if they have the same sequence of amino acids or nucleotides over the region being compared. Two sequences are “substantially identical” if two sequences have a specified percentage of amino acid residues or nucleotides that are the same (i.e., 60% identity, optionally 65%, 70%, 75%, 80%, 85%, 90%, 95%, or 99% identity over a specified region, or, when not specified, over the entire sequence), when compared and aligned for maximum correspondence over a comparison window, or designated region as measured using one of the following sequence comparison algorithms or by manual alignment and visual inspection. Optionally, the identity exists over a region that is at least about 30 nucleotides (or 10 amino acids) in length, or more preferably over a region that is 100 to 500 or 1000 or more nucleotides (or 20, 50, 200 or more amino acids) in length.

[0059] For sequence comparison, typically one sequence acts as a reference sequence, to which test sequences are compared. When using a sequence comparison algorithm, test and reference sequences are entered into a computer, subsequence coordinates are designated, if necessary, and sequence algorithm program parameters are designated. Default program parameters can be used, or alternative parameters can be designated. The sequence comparison algorithm then calculates the percent sequence identities for the test sequences relative to the reference sequence, based on the program parameters.

[0060] A “comparison window”, as used herein, includes reference to a segment of any one of the number of contiguous positions selected from the group consisting of from 20 to 600, usually about 50 to about 200, more usually about 100 to about 150 in which a sequence may be compared to a reference sequence of the same number of contiguous positions after the two sequences are optimally aligned. Methods of alignment of sequences for comparison are well known in the art. Optimal alignment of sequences for comparison can be conducted, *e.g.*, by the local homology algorithm of Smith and Waterman, Adv. Appl. Math. 2:482c (1970), by the homology alignment algorithm of Needleman and Wunsch, J. Mol. Biol. 48:443 (1970), by the search for similarity method of Pearson and Lipman, Proc. Natl. Acad. Sci. USA 85:2444 (1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Dr., Madison,

WI), or by manual alignment and visual inspection (see, *e.g.*, Brent *et al.*, Current Protocols in Molecular Biology, 2003).

[0061] Two examples of algorithms that are suitable for determining percent sequence identity and sequence similarity are the BLAST and BLAST 2.0 algorithms, which are described in Altschul *et al.*, Nuc. Acids Res. 25:3389-3402, 1977; and Altschul *et al.*, J. Mol. Biol. 215:403-410, 1990, respectively. Software for performing BLAST analyses is publicly available through the National Center for Biotechnology Information. This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying short words of length *W* in the query sequence, which either match or satisfy some positive-valued threshold score *T* when aligned with a word of the same length in a database sequence. *T* is referred to as the neighborhood word score threshold (Altschul *et al.*, *supra*). These initial neighborhood word hits act as seeds for initiating searches to find longer HSPs containing them. The word hits are extended in both directions along each sequence for as far as the cumulative alignment score can be increased. Cumulative scores are calculated using, for nucleotide sequences, the parameters *M* (reward score for a pair of matching residues; always > 0) and *N* (penalty score for mismatching residues; always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative score. Extension of the word hits in each direction are halted when: the cumulative alignment score falls off by the quantity *X* from its maximum achieved value; the cumulative score goes to zero or below, due to the accumulation of one or more negative-scoring residue alignments; or the end of either sequence is reached. The BLAST algorithm parameters *W*, *T*, and *X* determine the sensitivity and speed of the alignment. The BLASTN program (for nucleotide sequences) uses as defaults a word length (*W*) of 11, an expectation (*E*) or 10, *M*=5, *N*=-4 and a comparison of both strands. For amino acid sequences, the BLASTP program uses as defaults a word length of 3, and expectation (*E*) of 10, and the BLOSUM62 scoring matrix (see Henikoff and Henikoff, (1989) Proc. Natl. Acad. Sci. USA 89:10915) alignments (*B*) of 50, expectation (*E*) of 10, *M*=5, *N*=-4, and a comparison of both strands.

[0062] The BLAST algorithm also performs a statistical analysis of the similarity between two sequences (see, *e.g.*, Karlin and Altschul, Proc. Natl. Acad. Sci. USA 90:5873-5787, 1993). One measure of similarity provided by the BLAST algorithm is the smallest sum probability (*P(N)*), which provides an indication of the probability by which a match between two nucleotide or amino acid sequences would occur by chance. For example, a nucleic acid is

considered similar to a reference sequence if the smallest sum probability in a comparison of the test nucleic acid to the reference nucleic acid is less than about 0.2, more preferably less than about 0.01, and most preferably less than about 0.001.

[0063] The percent identity between two amino acid sequences can also 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 can be determined using the Needleman and Wunsch, *J. Mol. Biol.* 48:444-453, 1970) algorithm which has been incorporated into the GAP program in the GCG software package (available at www.gcg.com), using either a Blossom 62 matrix or a PAM250 matrix, and a gap weight of 16, 14, 12, 10, 8, 6, or 4 and a length weight of 1, 2, 3, 4, 5, or 6.

[0064] Other than percentage of sequence identity noted above, another indication that two nucleic acid sequences or polypeptides are substantially identical is that the polypeptide encoded by the first nucleic acid is immunologically cross reactive with the antibodies raised against the polypeptide encoded by the second nucleic acid, as described below. Thus, a polypeptide is typically substantially identical to a second polypeptide, for example, where the two peptides differ only by conservative substitutions. Another indication that two nucleic acid sequences are substantially identical is that the two molecules or their complements hybridize to each other under stringent conditions, as described below. Yet another indication that two nucleic acid sequences are substantially identical is that the same primers can be used to amplify the sequence.

[0065] The term “nucleic acid” is used herein interchangeably with the term “polynucleotide” and refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. The term encompasses nucleic acids containing known nucleotide analogs or modified backbone residues or linkages, which are synthetic, naturally occurring, and non-naturally occurring, which have similar binding properties as the reference nucleic acid, and which are metabolized in a manner similar to the reference nucleotides. Examples of such analogs include, without limitation, phosphorothioates, phosphoramidates, methyl phosphonates, chiral-methyl phosphonates, 2-O-methyl ribonucleotides, peptide-nucleic acids (PNAs).

[0066] Unless otherwise indicated, a particular nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.*, degenerate codon substitutions) and complementary sequences, as well as the sequence explicitly indicated. Specifically, as detailed below, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*, (1991) Nucleic Acid Res. 19:5081; Ohtsuka *et al.*, (1985) J. Biol. Chem. 260:2605-2608; and Rossolini *et al.*, (1994) Mol. Cell. Probes 8:91-98).

[0067] The term “operably linked” in the context of nucleic acids refers to a functional relationship between two or more polynucleotide (*e.g.*, DNA) segments. Typically, it refers to the functional relationship of a transcriptional regulatory sequence to a transcribed sequence. For example, a promoter or enhancer sequence is operably linked to a coding sequence if it stimulates or modulates the transcription of the coding sequence in an appropriate host cell or other expression system. Generally, promoter transcriptional regulatory sequences that are operably linked to a transcribed sequence are physically contiguous to the transcribed sequence, *i.e.*, they are *cis*-acting. However, some transcriptional regulatory sequences, such as enhancers, need not be physically contiguous or located in close proximity to the coding sequences whose transcription they enhance.

[0068] The terms “polypeptide” and “protein” are used interchangeably herein to refer to a polymer of amino acid residues. The terms apply to amino acid polymers in which one or more amino acid residue is an artificial chemical mimetic of a corresponding naturally occurring amino acid, as well as to naturally occurring amino acid polymers and non-naturally occurring amino acid polymer. Unless otherwise indicated, a particular polypeptide sequence also implicitly encompasses conservatively modified variants thereof.

[0069] The term “immunoconjugate” or “antibody drug conjugate” or “conjugate” as used herein refers to the linkage of an antibody or an antigen binding fragment thereof with another agent, such as a chemotherapeutic agent, a toxin, an immunotherapeutic agent, an imaging probe, and the like. The linkage can be covalent bonds, or non-covalent interactions such as through electrostatic forces. Various linkers, known in the art, can be employed in order to form the immunoconjugate. Additionally, the immunoconjugate can be provided in the form of a fusion protein that may be expressed from a polynucleotide encoding the immunoconjugate. As used herein, “fusion protein” refers to proteins created through the joining of two or more genes or

gene fragments which originally coded for separate proteins (including peptides and polypeptides). Translation of the fusion gene results in a single protein with functional properties derived from each of the original proteins.

[0070] The term “salt” or “salts” refers to an acid addition or base addition salt of a compound, for example, a compound which is another therapeutic agent administered in combination with the antibody drug conjugate of the invention. “Salts” include in particular “pharmaceutically acceptable salts”. The term “pharmaceutically acceptable salts” refers to salts that retain the biological effectiveness and properties of the compound and, which typically are not biologically or otherwise undesirable. In many cases, the compound is capable of forming acid and/or base salts by virtue of the presence of amino and/or carboxyl groups or groups similar thereto.

[0071] The term “subject” includes human and non-human animals. Non-human animals include all vertebrates, *e.g.*, mammals and non-mammals, such as non-human primates, sheep, dog, cow, chickens, amphibians, and reptiles. Except when noted, the terms “patient” or “subject” are used herein interchangeably.

[0072] The term “cytotoxin”, or “cytotoxic agent” as used herein, refers to any agent that is detrimental to the growth and proliferation of cells and may act to reduce, inhibit, or destroy a cell or malignancy.

[0073] The term “anti-cancer agent” as used herein refers to any agent that can be used to treat a cell proliferative disorder such as cancer, including but not limited to, cytotoxic agents, chemotherapeutic agents, radiotherapy and radiotherapeutic agents, targeted anti-cancer agents, and immunotherapeutic agents.

[0074] The term “drug moiety” or “payload” as used herein refers to a chemical moiety that is conjugated to an antibody or antigen binding fragment of the invention, and can include any therapeutic or diagnostic agent, for example, an anti-cancer, anti-inflammatory, anti-infective (*e.g.*, anti-fungal, antibacterial, anti-parasitic, anti-viral), or an anesthetic agent. For example, the drug moiety can be an anti-cancer agent, such as a cytotoxin. In certain embodiments, a drug moiety is selected from a V-ATPase inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, an inhibitor of phosphoryl transfer reactions in mitochondria, a protein

synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a proteasome inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor. Methods for attaching each of these to a linker compatible with the antibodies and method of the invention are known in the art. See, e.g., Singh et al., (2009) *Therapeutic Antibodies: Methods and Protocols*, vol. 525, 445-457. In addition, a payload can be a biophysical probe, a fluorophore, a spin label, an infrared probe, an affinity probe, a chelator, a spectroscopic probe, a radioactive probe, a lipid molecule, a polyethylene glycol, a polymer, a spin label, DNA, RNA, a protein, a peptide, a surface, an antibody, an antibody fragment, a nanoparticle, a quantum dot, a liposome, a PLGA particle, a saccharide or a polysaccharide.

[0075] The term "maytansinoid drug moiety" means the substructure of an antibody-drug conjugate that has the structure of a maytansinoid compound. Maytansine was first isolated from the east African shrub *Maytenus serrata* (U.S. Pat. No. 3,896,111). Subsequently, it was discovered that certain microbes also produce maytansinoids, such as maytansinol and C-3 maytansinol esters (U.S. Pat. No. 4,151,042). Synthetic maytansinol and maytansinol analogues have been reported. See U.S. Pat. Nos. 4,137,230; 4,248,870; 4,256,746; 4,260,608; 4,265,814; 4,294,757; 4,307,016; 4,308,268; 4,308,269; 4,309,428; 4,313,946; 4,315,929; 4,317,821; 4,322,348; 4,331,598; 4,361,650; 4,364,866; 4,424,219; 4,450,254; 4,362,663; and 4,371,533, and Kawai et al (1984) *Chem. Pharm. Bull.* 3441-3451), each of which are expressly incorporated by reference. Examples of specific maytansinoids useful for conjugation include DM1, DM3 and DM4.

[0076] "Tumor" refers to neoplastic cell growth and proliferation, whether malignant or benign, and all pre-cancerous and cancerous cells and tissues.

[0077] The term "anti-tumor activity" means a reduction in the rate of tumor cell proliferation, viability, or metastatic activity. For example, anti-tumor activity can be shown by a decline in growth rate of abnormal cells that arises during therapy or tumor size stability or reduction, or longer survival due to therapy as compared to control without therapy. Such activity can be assessed using accepted in vitro or in vivo tumor models, including but not limited to xenograft models, allograft models, MMTV models, and other known models known in the art to investigate anti-tumor activity.

[0078] The term “malignancy” refers to a non-benign tumor or a cancer. As used herein, the term “cancer” includes a malignancy characterized by deregulated or uncontrolled cell growth. Exemplary cancers include: carcinomas, sarcomas, leukemias, and lymphomas.

[0079] The term “cancer” includes primary malignant tumors (*e.g.*, those whose cells have not migrated to sites in the subject's body other than the site of the original tumor) and secondary malignant tumors (*e.g.*, those arising from metastasis, the migration of tumor cells to secondary sites that are different from the site of the original tumor).

[0080] The term “FGFR2” refers to fibroblast growth factor receptor 2 that is a member of the receptor tyrosine kinase superfamily. The nucleic acid and amino acid sequences of FGFR2 are known, and have been published in GenBank Accession Nos. NM_000141.4, NM_001144913.1, NM_001144914.1, NM_001144915.1, NM_001144916.1, NM_001144917.1, NM_001144918.1, NM_001144919.1, NM_022970.3, NM_023029.2. Structurally, a FGFR2 amino acid sequence is a receptor tyrosine kinase protein having a signal peptide that is cleaved off, at least one or more immunoglobulin (Ig)-like domains, an acidic box, a transmembrane domain, and a split tyrosine kinase domain and has over its full length at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the amino acid sequence of GenBank accession numbers NM_000141.4, NM_001144913.1, NM_001144914.1, NM_001144915.1, NM_001144916.1, NM_001144917.1, NM_001144918.1, NM_001144919.1, NM_022970.3, NM_023029.2. Structurally, a FGFR2 nucleic acid sequence has over its full length at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the nucleic acid sequence of GenBank accession numbers NM_000141.4, NM_001144913.1, NM_001144914.1, NM_001144915.1, NM_001144916.1, NM_001144917.1, NM_001144918.1, NM_001144919.1, NM_022970.3, NM_023029.2.

[0081] The term “FGFR4” refers to fibroblast growth factor receptor 4 that is a member of the receptor tyrosine kinase superfamily. The nucleic acid and amino acid sequences of FGFR4 are known, and have been published in GenBank Accession Nos. NM_002011.3, NM_022963.2, NM_213647.1. Structurally, a FGFR4 amino acid sequence is a receptor tyrosine kinase protein having a signal peptide that is cleaved off, three immunoglobulin (Ig)-like domains, an acidic box, a transmembrane domain, and a split tyrosine kinase domain and has over its full length at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the amino acid sequence of GenBank accession numbers NM_002011.3,

NM_022963.2, NM_213647.1. Structurally, a FGFR4 nucleic acid sequence has over its full length at least about 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity with the nucleic acid sequence of GenBank accession numbers NM_002011.3, NM_022963.2, NM_213647.1.

[0082] The term “FGFR2/4” is used herein to refer to FGFR2 and FGFR4. For example, antibodies that bind FGFR2/4 refers to antibodies that bind FGFR2 and FGFR4.

[0083] The terms “FGFR2 expressing cancer” or “FGFR2 positive cancer” refers to a cancer that express FGFR2 or a mutation form of FGFR2 on the surface of cancer cells. The terms “FGFR4 expressing cancer” or “FGFR4 positive cancer” refers to a cancer that express FGFR4 or a mutation form of FGFR4 on the surface of cancer cells.

[0084] The term “tyrosine kinase resistant cancer” or “tyrosine kinase resistant tumor” refers to (1) a cancer or a tumor that does not respond to a standard of care tyrosine kinase treatment to begin with and is referred to as “*de novo* resistant;” or (2) a cancer or a tumor which initially responded to standard of care tyrosine kinase treatment and progressing thereafter as determined by reemergence of old and/or new lesions or spreading, which are also referred to as “secondary resistant” or “relapsed cancer .”

[0085] The term “Her2 resistant cancer” or “Her2 resistant tumor” refers to (1) a cancer or a tumor that does not respond to a standard of care Her 2 treatment to begin with and is referred to as “*de novo* resistant;” or (2) a cancer or a tumor which initially responded to standard of care Her2 treatment and progressing thereafter as determined by reemergence of old and/or new lesions or spreading, which are also referred to as “secondary resistant” or “relapsed cancer .”

[0086] As used herein, the terms “treat,” “treating,” or “treatment” of any disease or disorder refers in one embodiment, to ameliorating the disease or disorder (i.e., slowing or arresting or reducing the development of the disease or at least one of the clinical symptoms thereof). In another embodiment, “treat,” “treating,” or “treatment” refers to alleviating or ameliorating at least one physical parameter including those which may not be discernible by the patient. In yet another embodiment, “treat,” “treating,” or “treatment” refers to modulating the disease or disorder, either physically, (e.g., stabilization of a discernible symptom), physiologically, (e.g., stabilization of a physical parameter), or both. In yet another embodiment, “treat,” “treating,” or “treatment” refers to delaying the onset or development or progression of the disease or disorder.

[0087] The term “therapeutically acceptable amount” or “therapeutically effective dose” interchangeably refers to an amount sufficient to effect the desired result (i.e., a reduction in tumor size, inhibition of tumor growth, prevention of metastasis, inhibition or prevention of viral, bacterial, fungal or parasitic infection). In some embodiments, a therapeutically acceptable amount does not induce or cause undesirable side effects. In some embodiments, a therapeutically acceptable amount induces or causes side effects but only those that are acceptable by the healthcare providers in view of a patient’s condition. A therapeutically acceptable amount can be determined by first administering a low dose, and then incrementally increasing that dose until the desired effect is achieved. A “prophylactically effective dosage,” and a “therapeutically effective dosage,” of the molecules of the invention can prevent the onset of, or result in a decrease in severity of, respectively, disease symptoms, including symptoms associated with cancer.

[0088] The term “co-administer” refers to the presence of two active agents in the blood of an individual. Active agents that are co-administered can be concurrently or sequentially delivered.

BRIEF DESCRIPTION OF THE DRAWINGS

[0089] FIG. 1 shows affinity estimates for a panel of antibodies to recombinant human FGFR2 IIIb.

[0090] FIG. 2 (A) - (B) show the ability of anti-FGFR antibodies to act as agonists in a Baf-FGFR2 receptor system.

[0091] FIG. 3 (A) – (B) show the ability of anti-FGFR antibodies to act as antagonists in a Baf-FGFR2 receptor system;

[0092] FIG. 4 (A) – (B) show the anti-tumor activity of anti-FGFR2- and anti-FGFR2/4-MCC-DM1 ADCs in a SNU16 tumor xenograft mouse model; (C) shows the anti-tumor activity of the 12433 antibody as an ADC conjugated to SMCC-DM1 and SPDB-DM4 linker-payloads; (D) – (E) show the pharmacokinetic properties of anti-FGFR2- and anti-FGFR2/4-MCC-DM1 ADCs; (F) shows the anti-tumor activity against ADC clearance for a panel of anti-FGFR2- and anti-FGFR2/4-MCC-DM1 ADCs.

[0093] FIG. 5 (A) – (C) are western blots showing the ability of anti-FGFR antibodies or ADCs to modulate FGFR signaling and total receptor expression in FGFR2-amplified cell lines, SNU16 after 2 hours (A), 2-72 hours (B) or Kato-III cells (C).

[0094] FIG. 6 (A) – (D) show the ability of 12425-MCC-DM1 to inhibit the proliferation of SNU-16 (A), Kato-III (B) or NUGC3 (C) cells relative to IgG and free payload controls; (D) is shows that the unconjugated antibody, 12425, did not have any appreciable anti-proliferative effect.

[0095] FIG. 7 (A) – (B) are images of SNU-16 (A) or NUGC3 (B) tumor xenografts following treatment with 12425-MCC-DM1 that show the assessment of pHH3 and cleaved caspase 3 expression.

[0096] FIG. 8 (A) – (F) show the anti-tumor activity of anti-FGFR2- and/or anti-FGFR2/4-MCC-DM1 ADCs in NCI-H716 (A), MFM223 (B), a human primary gastric tumor xenograph (CHGA-010) (C), a human primary lung tumor xenograph model (HLUX1228) (D), a human primary breast cancer xenograph model (E) and a human primary breast ductal carcinoma xenograph model (F).

[0097] FIG. 9 shows the anti-tumor activity of the anti FGFR2/4 ADC, 12425-MCC-DM1, alone and in combination with the FGFR tyrosine kinase inhibitor, BGJ398 in the CHGA-119 tumor xenograft model.

[0098] FIG. 10 shows the anti-tumor activity of the anti FGFR2/4 ADC, 12425-MCC-DM1 at different doses and schedules in the SNU-16 tumor xenograft mouse model.

[0099] FIG. 11 (A) – (C) show the assessment of the the ability of anti-FGFR2 antibodies to induce ADCC in vitro in Kato III cells (A), to bind to C1q (B), induce CDC (C) in Kato III cells. (D) shows the effect of an ADCC depleted variant as an unconjugated antibody or MCC-DM1 conjugated ADC in vivo.

[00100] FIG. 12 (A) – (B) are western blots showing the ability of anti-FGFR antibodies or ADCs to modulate FGFR signaling and total receptor expression in the FGFR4-expressing cell line, MDA-MB453 after 2 hours (A) or over a time course (B).

[00101] FIG. 13 (A) – (D) show the ability of FGFR2 or FGFR2/4 antibodies (unconjugated or as MCC-DM1 ADCs) to inhibit the proliferation MDA-MB453 (A), RH4 (B), JR cells (C) or a cancer cell line panel (D).

[00102] FIG. 14 (A) – (C) are images of MDA-MB453 ((A) and (B)) or RH4 (C) tumor xenografts following treatment with 12425-MCC-DM1 that show the assessment of pHH3 and/or cleaved caspase 3 expression.

[00103] FIG. 15 (A) – (B) show the anti-tumor activity of anti-FGFR2- and anti-FGFR2/4-MCC-DM1 ADCs in MDA-MB453 (A), and RH4 (B) tumor xenograft mouse models.

[00104] FIG. 16 (A) – (D) show the PK profiles of 12425-MCC-DM1 in mouse ((A) – (B)), rat (C), or cynomolgus monkey (D).

[00105] FIG. 17 (A) – (B) show the ability of the engineered variants of 12425 (20562-MCC-DM1) to inhibit the proliferation of SNU-16 cells *in vitro* (A) and *in vivo* (B).

[00106] FIG. 18 (A) – (B) show the ability of the affinity-matured variants of 10164 (20809-MCC-DM1) and 12425 (20811-MCC-DM1) to inhibit the proliferation of SNU-16 cells *in vitro* (A) and *in vivo* (B).

[00107] FIG. 19 shows the results of deuterium exchange experiments to FGFR2. (A) shows the absolute deuterium uptake for FGFR2 in the absence (control) and presence of six therapeutic mAb. The heights of the bars are the average of three measurements and the error bars are one standard deviation; (B) shows the difference in deuterium uptake between mAb bound and control FGFR2 divided by the standard error in the measurement; (C) shows regions of high protection upon binding of mAbs to FGFR2 mapped onto FGFR2 IIIc: FGFR2 crystal structure (PDB ID: 1EV2); and (D) shows regions of high protection upon binding of mAbs to FGFR2 mapped onto FGFR2 IIIb: FGFR1 crystal structure (PDB ID: 3OJM).

[00108] FIG. 20 shows the results from X-ray crystallography epitope mapping studies. (A) shows the overall structure of 12425 Fab binding to FGFR2 (left panel) and detailed interaction surface on FGFR2 with epitope residues labeled (right panel); (B) shows the overall structure of 12425 Fab binding to FGFR4 (left panel) and detailed interaction surface on FGFR4 with epitope residues labeled (right panel); and (C) shows a sequence alignment of human FGFR2 and FGFR4 in the D2 domain. Completely conserved residues are shaded in dark grey, partially conserved residues in light grey. Dashed boxes are FGFR2 residues contacting 12425 Fab, along with their aligned counterparts in FGFR4.

[00109] FIG.21 Shows the results of proliferation stides comparing the sensitivity of SNU 16 cells and BGJ398 resistance SNU 16 cells to BGJ398 or Gefitinib ($IC_{50} = 16.7$ nM) *in vitro*.

DETAILED DESCRIPTION OF THE INVENTION

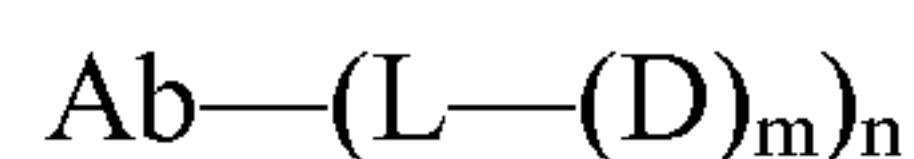
[00110] The present invention provides antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates that bind to both FGFR2 and FGFR4 (“FGFR2/4”). In particular, the present invention provides antibodies and antibody fragments (*e.g.*, antigen binding fragments) that bind to FGFR2/4, and internalize upon such binding. The antibodies and antibody fragments (*e.g.*, antigen binding fragments) of the present invention can be used for producing antibody drug conjugates. Furthermore, the present invention provides antibody drug conjugates that have desirable pharmacokinetic characteristics and other desirable

attributes, and thus can be used for treating cancer expressing FGFR2 and/or FGFR4, such as gastric cancer, breast cancer, rhabdomyosarcoma (e.g.: alveolar rhabdomyosarcoma or embryonal rhabdomyosarcoma), liver cancer, adrenal cancer, lung cancer, colon cancer, and endometrial cancer. The present invention further provides pharmaceutical compositions comprising the antibody drug conjugates of the invention, and methods of making and using such pharmaceutical compositions for the treatment of cancer.

Antibody Drug Conjugates

[00111] The present invention provides antibody drug conjugates, where an antibody, antigen binding fragment or its functional equivalent that specifically binds to FGFR2/4 is linked to a drug moiety. In one aspect, the antibodies, antigen binding fragments or their functional equivalents of the invention are linked, via covalent attachment by a linker, to a drug moiety that is an anti-cancer agent. The antibody drug conjugates of the invention can selectively deliver an effective dose of an anti-cancer agent (e.g., a cytotoxic agent) to tumor tissues expressing FGFR2 and/or FGFR4, whereby greater selectivity (and lower efficacious dose) may be achieved.

[00112] In one aspect, the invention provides an immunoconjugate of Formula (I):



Wherein Ab represents an FGFR2/4 binding antibody described herein;

L is a linker;

D is a drug moiety;

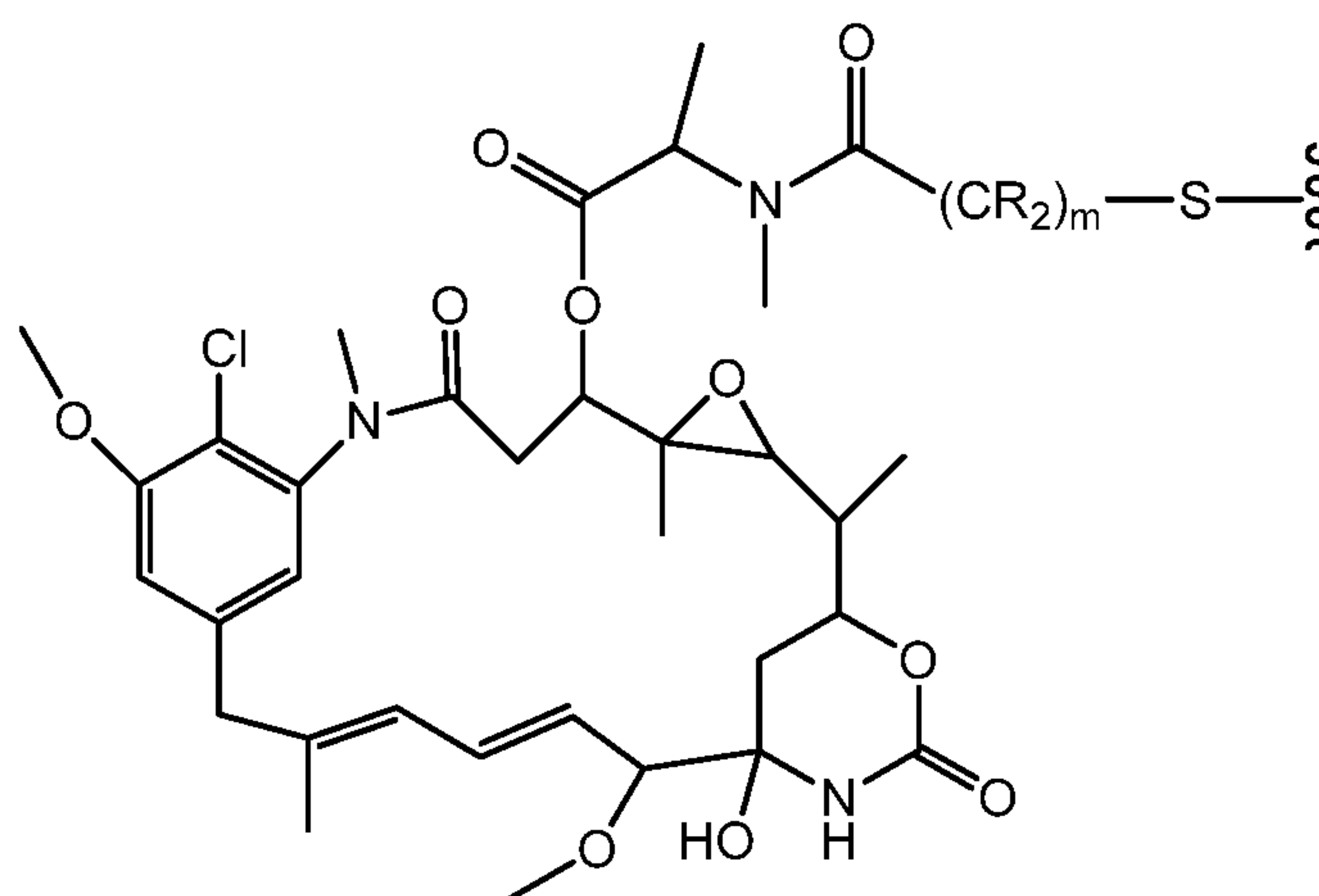
m is an integer from 1 to 8; and

n is an integer from 1-20. In one embodiment, n is an integer from 1 to 10, 2 to 8, or 2 to 5. In a specific embodiment, n is 2, 3, or 4. In some embodiments, m is 1; in other embodiments m is 2, 3 or 4.

[00113] While the drug to antibody ratio has an exact value for a specific conjugate molecule (e.g., n multiplied by m in Formula (I)), it is understood that the value will often be an average value when used to describe a sample containing many molecules, due to some degree of inhomogeneity, typically associated with the conjugation step. The average loading for a sample of an immunoconjugate is referred to herein as the drug to antibody ratio, or “DAR.” In some embodiments, when the drug is maytansinoid, it is referred to as “MAR.” In some embodiments, the DAR is between about 1 and about 5, and typically is about 3, 3.5, 4, 4.5, or 5. In some embodiments, at least 50% of a sample by weight is compound having the average DAR plus or

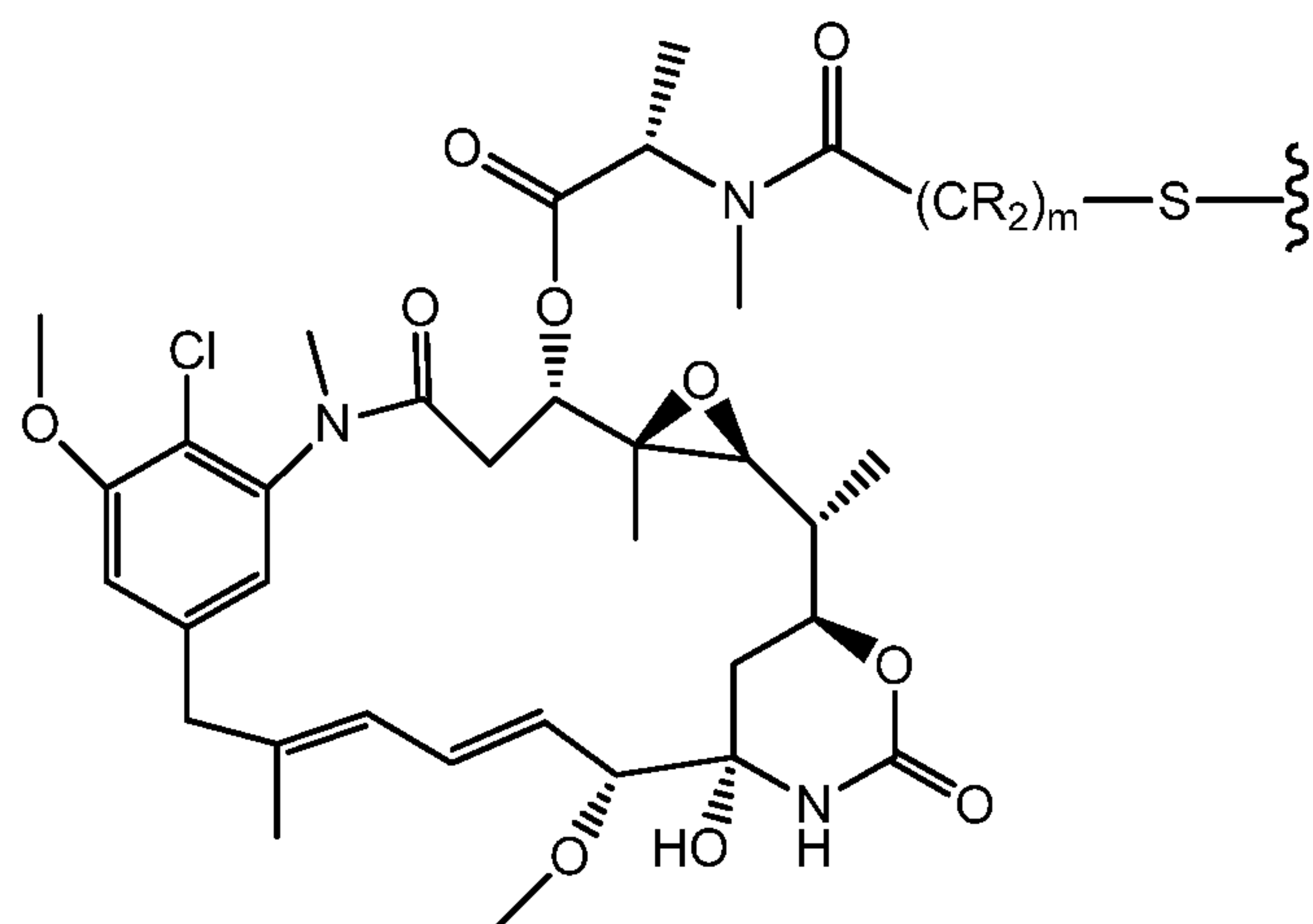
minus 2, and preferably at least 50% of the sample is a conjugate that contains the average DAR plus or minus 1. Preferred embodiments include immunoconjugates wherein the DAR is about 3.5. In some embodiments, a DAR of 'about n' means the measured value for DAR is within 20% of n.

[00114] The present invention is also directed to immunoconjugates comprising the antibodies, antibody fragments (*e.g.*, antigen binding fragments) and their functional equivalents as disclosed herein, linked or conjugated to a drug moiety. In one embodiment, the drug moiety D is a maytansinoid drug moiety, including those having the structure:

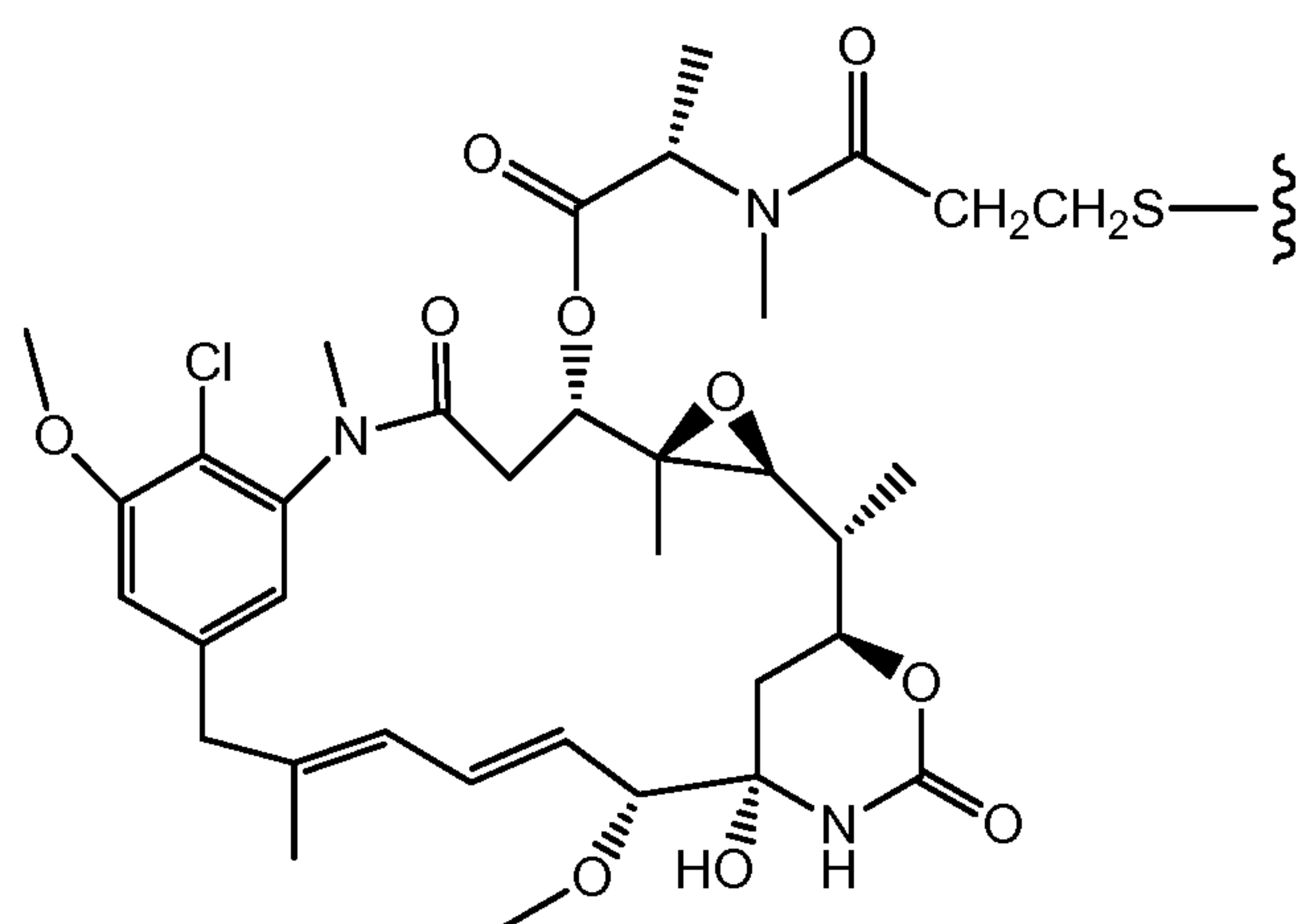


where the wavy line indicates the covalent attachment of the sulfur atom of the maytansinoid to a linker of an antibody drug conjugate. R at each occurrence is independently H or a C₁-C₆ alkyl. The alkylene chain attaching the amide group to the sulfur atom may be methanyl, ethanyl, or propyl, *i.e.* m is 1, 2, or 3. (U.S. Pat. No. 633,410, U.S. Pat. No. 5,208,020, Chari *et al.* (1992) Cancer Res. 52:127-131, Lui *et al.* (1996) Proc. Natl. Acad. Sci. 93:8618-8623).

[00115] All stereoisomers of the maytansinoid drug moiety are contemplated for the immunoconjugates of the invention, *i.e.* any combination of R and S configurations at the chiral carbons of the maytansinoid. In one embodiment the maytansinoid drug moiety has the following stereochemistry.

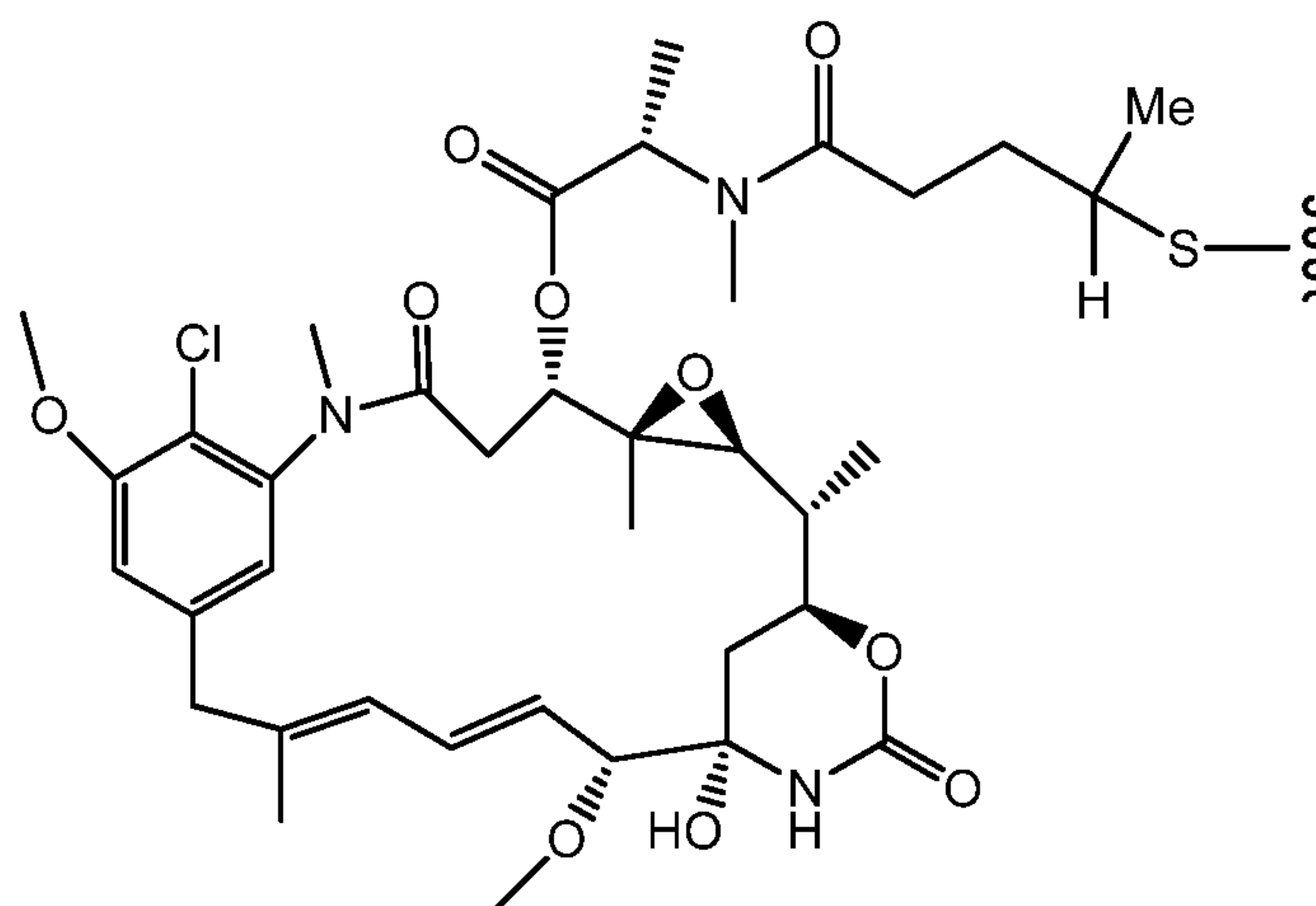


[00116] In one embodiment, the maytansinoid drug moiety is $N^{2'}$ -deacetyl- $N^{2'}$ -(3-mercapto-1-oxopropyl)-maytansine (also known as DM1). DM1 is represented by the following structural formula.



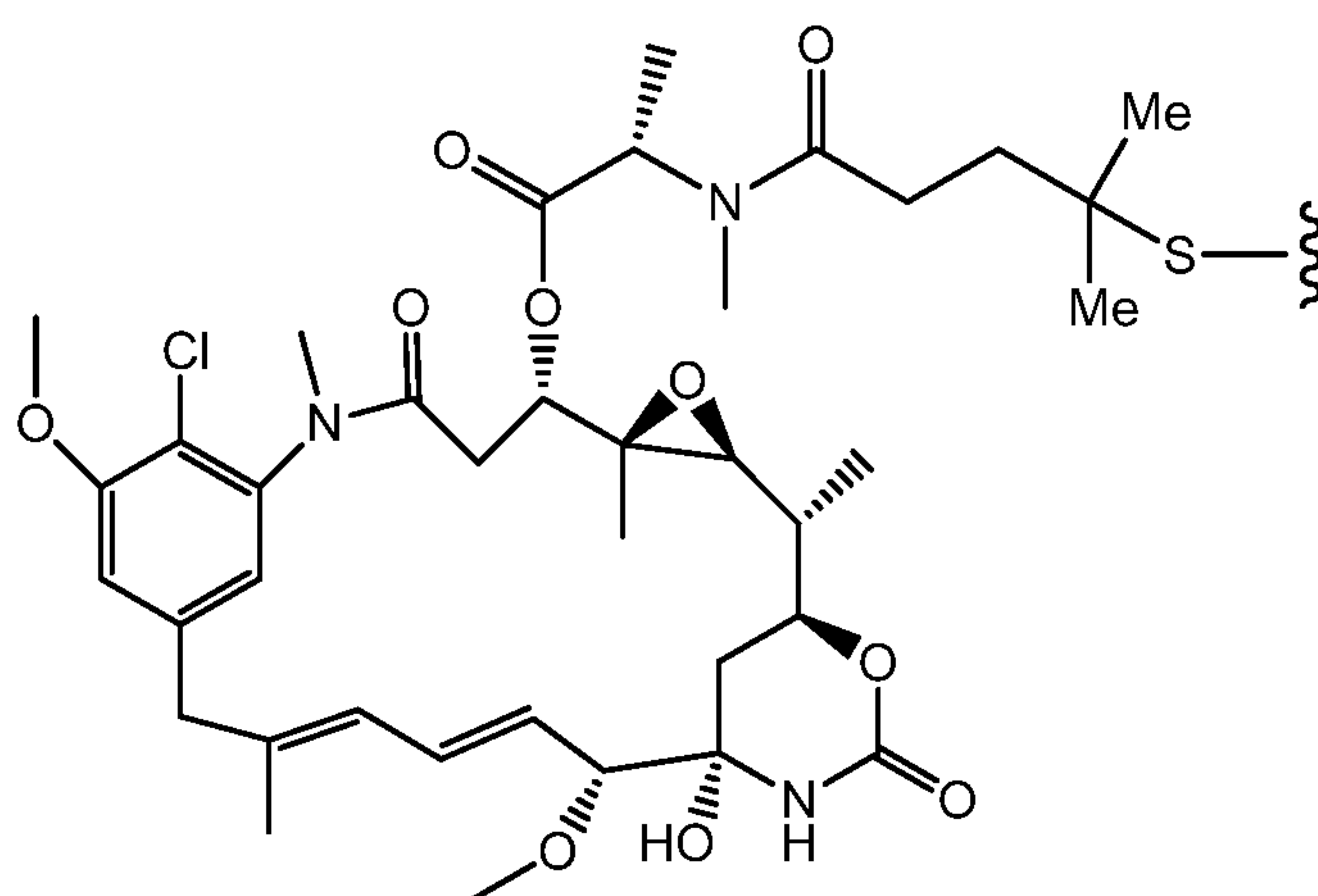
DM1

[00117] In another embodiment the maytansinoid drug moiety is $N^{2'}$ -deacetyl- $N^{2'}$ -(4-mercapto-1-oxopentyl)-maytansine (also known as DM3). DM3 is represented by the following structural formula.



DM3

[00118] In another embodiment the maytansinoid drug moiety is $N^{2'}$ -deacetyl- $N^{2'}$ -(4-methyl-4-mercapto-1-oxopentyl)-maytansine (also known as DM4). DM4 is represented by the following structural formula.



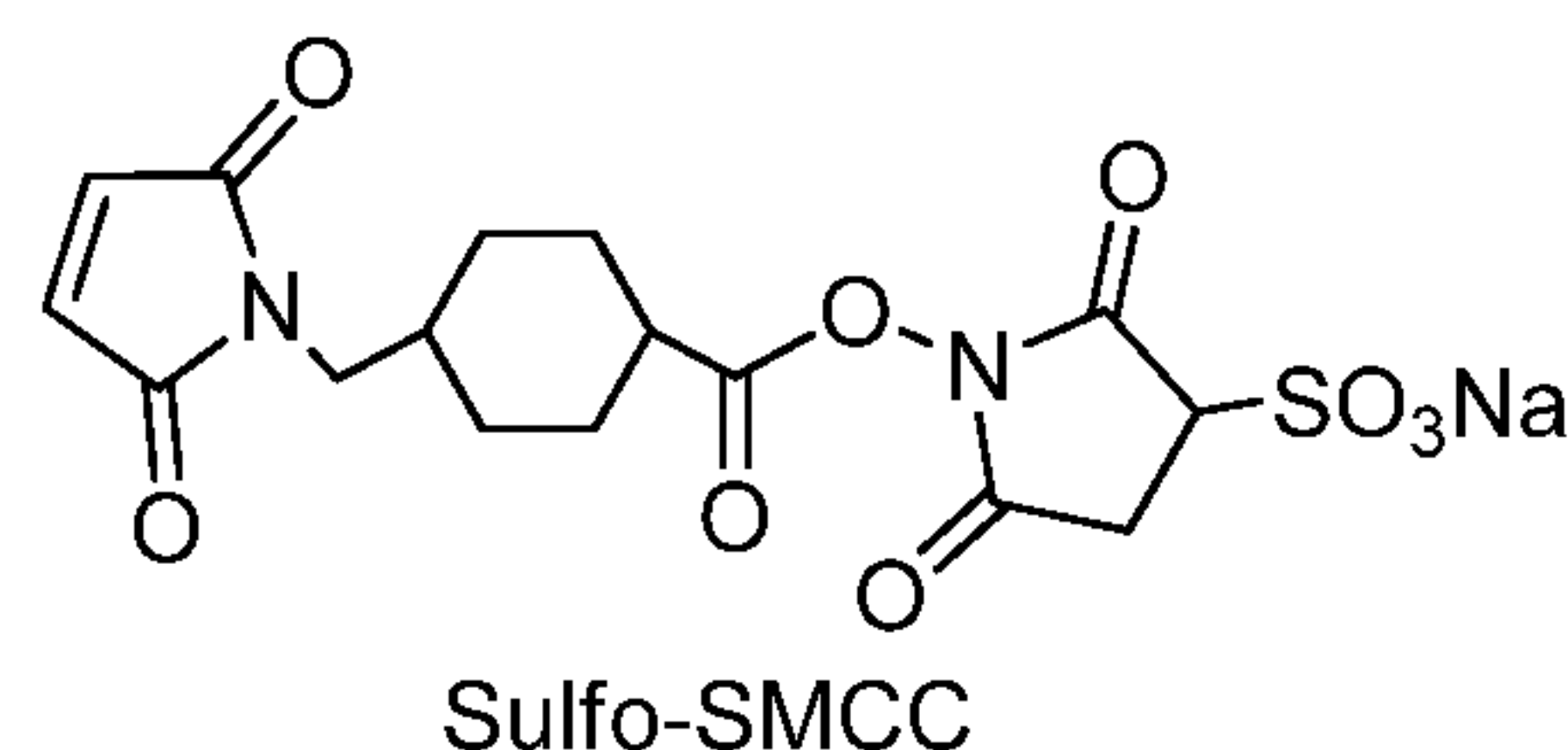
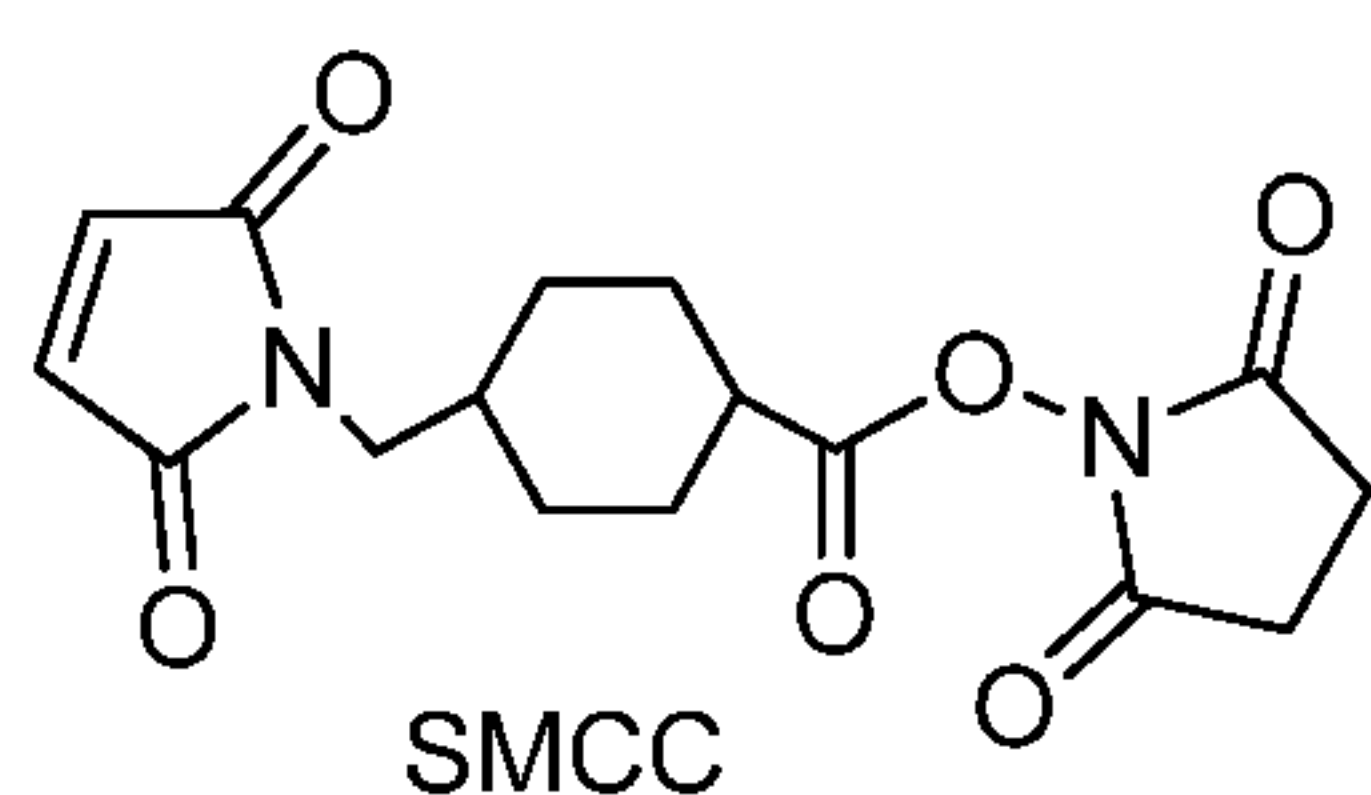
DM4

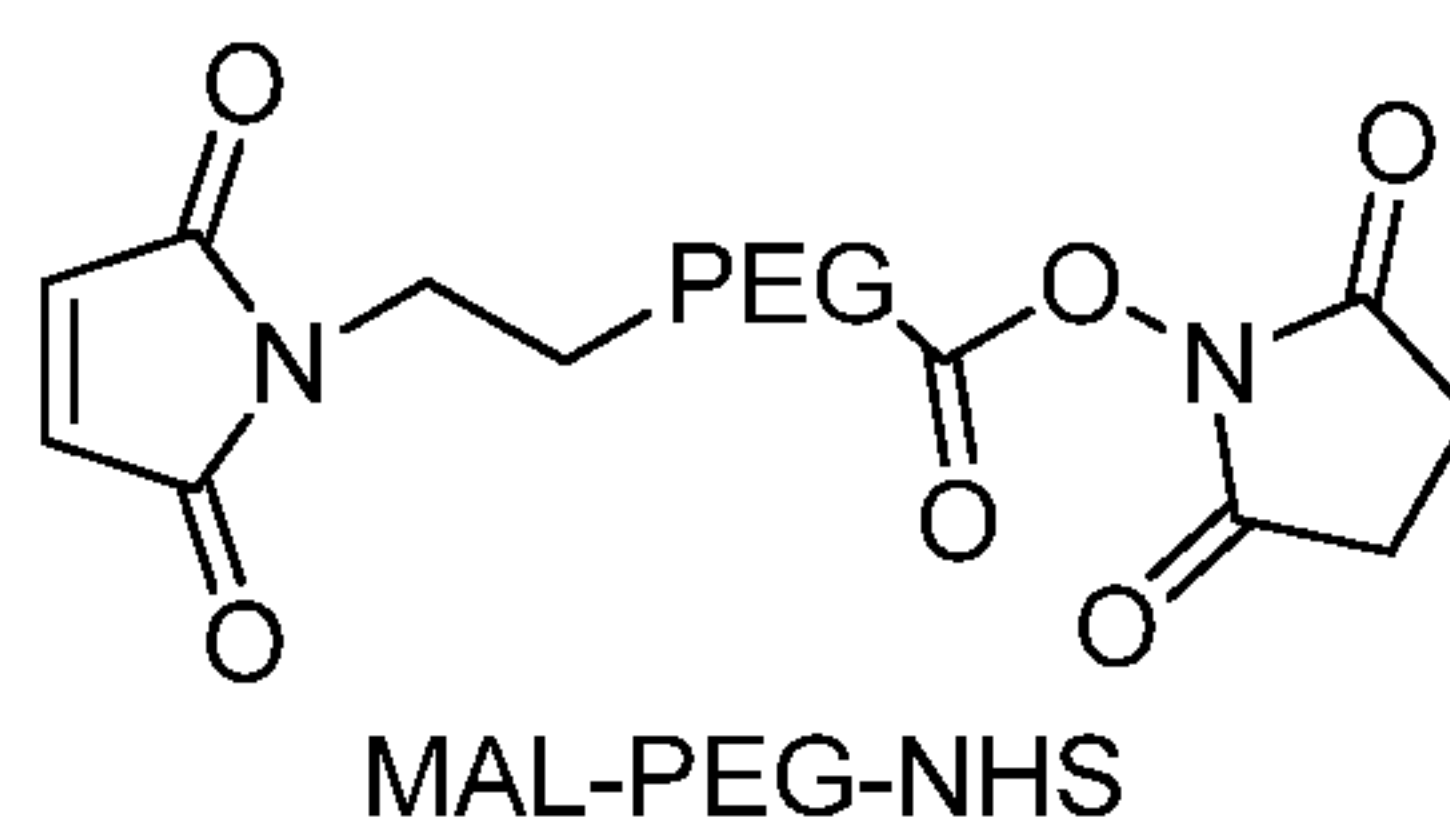
[00119] The drug moiety D can be linked to the antibody Ab through linker L. L is any chemical moiety capable of linking the drug moiety to the antibody through covalent bonds. A cross-linking reagent is a bifunctional or multifunctional reagent that can be used to link a drug moiety and an antibody to form an antibody drug conjugate. Antibody drug conjugates can be prepared using a cross-linking reagent having a reactive functionality capable of binding to both the drug moiety and the antibody. For example, a cysteine, thiol or an amine, e.g. N-terminus or an amino acid side chain, such as lysine of the antibody, can form a bond with a functional group of a cross-linking reagent.

[00120] In one embodiment, L is a cleavable linker. In another embodiment, L is a non-cleavable linker. In some embodiments, L is an acid-labile linker, a photo-labile linker, a peptidase cleavable linker, an esterase cleavable linker, a disulfide bond cleavable linker, a hydrophilic linker, a procharged linker, or a dicarboxylic acid based linker.

[00121] Suitable cross-linking reagents that form a non-cleavable linker between the drug moiety, for example maytansinoid, and the antibody are well known in the art, and can form non-cleavable linkers that comprise a sulfur atom (such as SMCC) or those that are without a sulfur atom. Preferred cross-linking reagents that form non-cleavable linkers between the drug moiety, for example maytansinoid, and the antibody comprise a maleimido- or haloacetyl-based moiety. According to the present invention, such non-cleavable linkers are said to be derived from maleimido- or haloacetyl-based moieties.

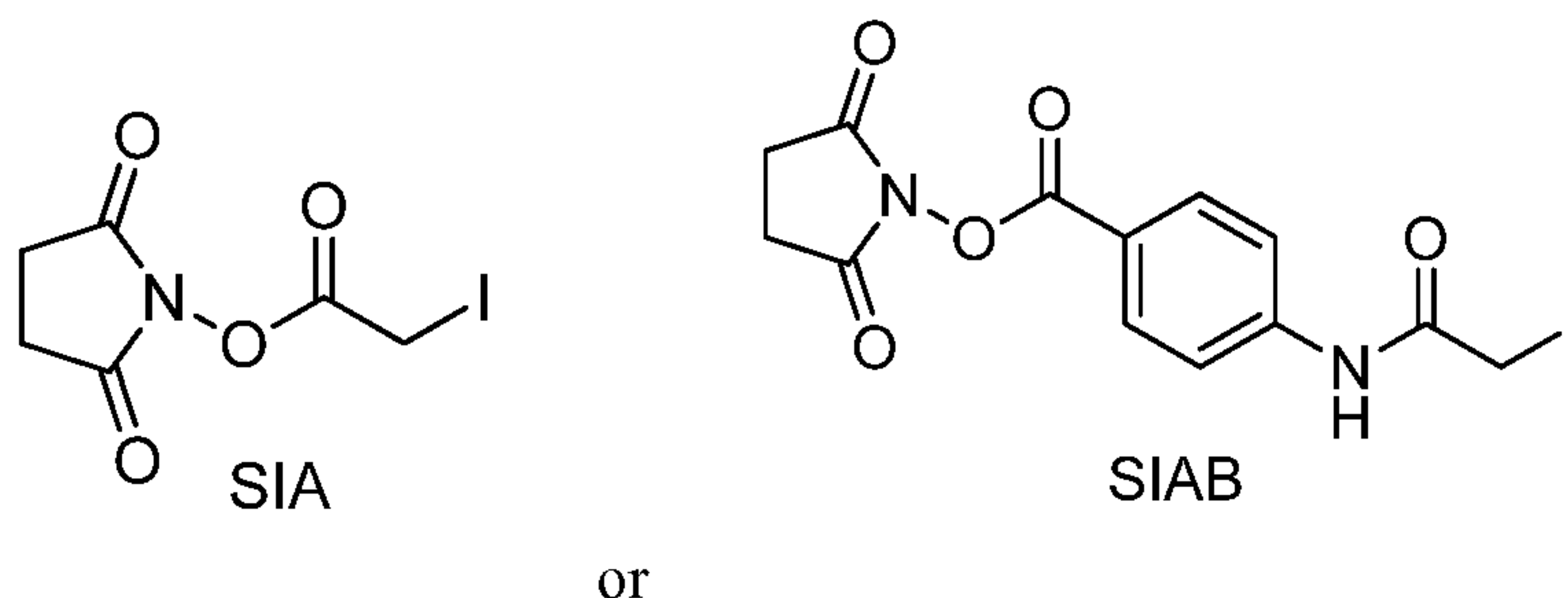
[00122] Cross-linking reagents comprising a maleimido-based moiety include but not limited to, *N*-succinimidyl-4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), sulfosuccinimidyl 4-(*N*-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC), *N*-succinimidyl-4-(maleimidomethyl)cyclohexane-1-carboxy-(6-amidocaproate), which is a “long chain” analog of SMCC (LC-SMCC), κ -maleimidoundeconoic acid *N*-succinimidyl ester (KMUA), γ -maleimidobutyric acid *N*-succinimidyl ester (GMBS), ϵ -maleimidocaproic acid *N*-hydroxysuccinimidyl ester (EMCS), *m*-maleimidobenzoyl-*N*-hydroxysuccinimide ester (MBS), *N*-(α -maleimidoacetoxy)-succinimide ester (AMSA), succinimidyl-6-(β -maleimidopropionamido)hexanoate (SMPH), *N*-succinimidyl-4-(*p*-maleimidophenyl)-butyrate (SMPB), *N*-(*p*-maleimidophenyl)isocyanate (PMIP) and maleimido-based cross-linking reagents containing a polyethylene glycol spacer, such as MAL-PEG-NHS. These cross-linking reagents form non-cleavable linkers derived from maleimido-based moieties. Representative structures of maleimido-based cross-linking reagents are shown below.





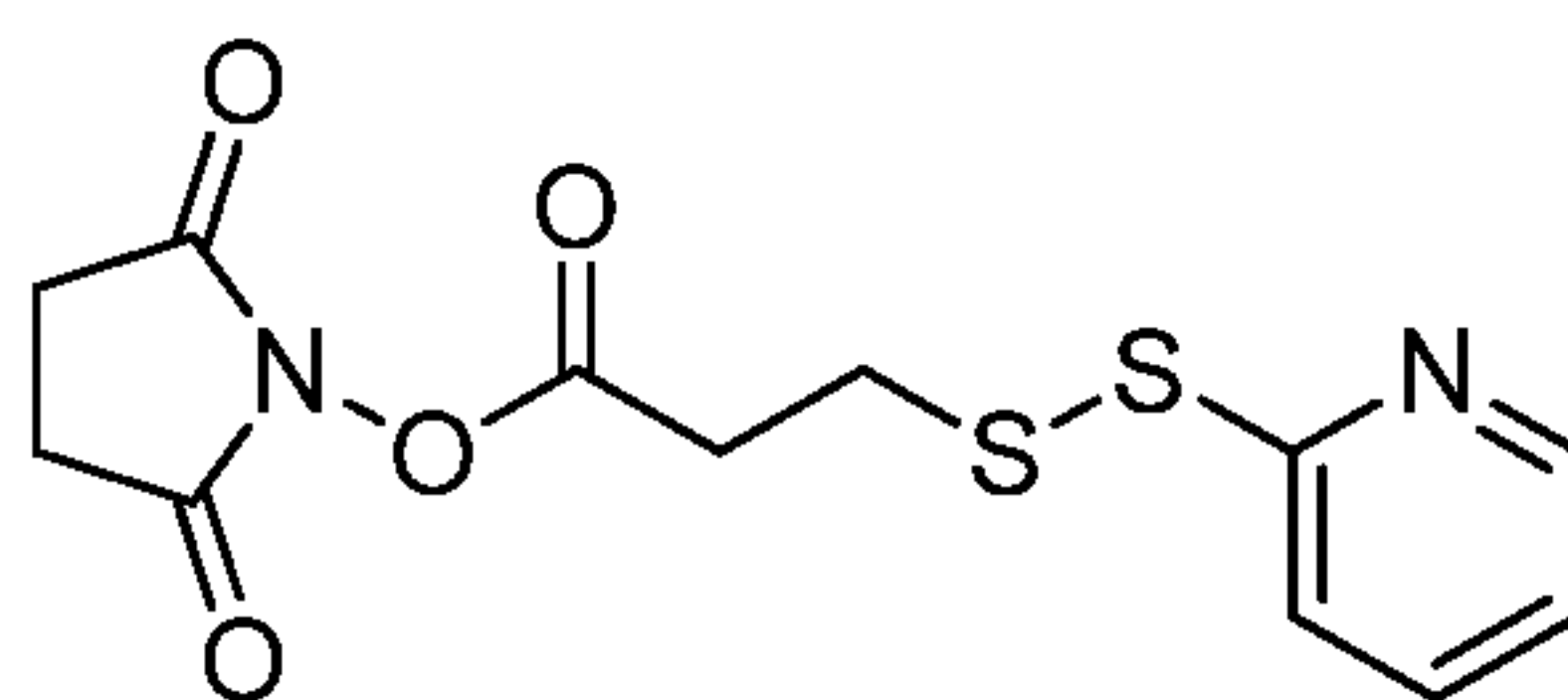
[00123] In another embodiment, the linker L is derived from *N*-succinimidyl-4-(maleimidomethyl)cyclohexanecarboxylate (SMCC), sulfosuccinimidyl 4-(*N*-maleimidomethyl)cyclohexane-1-carboxylate (sulfo-SMCC) or MAL-PEG-NHS.

[00124] Cross-linking reagents comprising a haloacetyl-based moiety include N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), N-succinimidyl bromoacetate (SBA) and N-succinimidyl 3-(bromoacetamido)propionate (SBAP). These cross-linking reagents form a non-cleavable linker derived from haloacetyl-based moieties. Representative structures of haloacetyl-based cross-linking reagents are shown below.

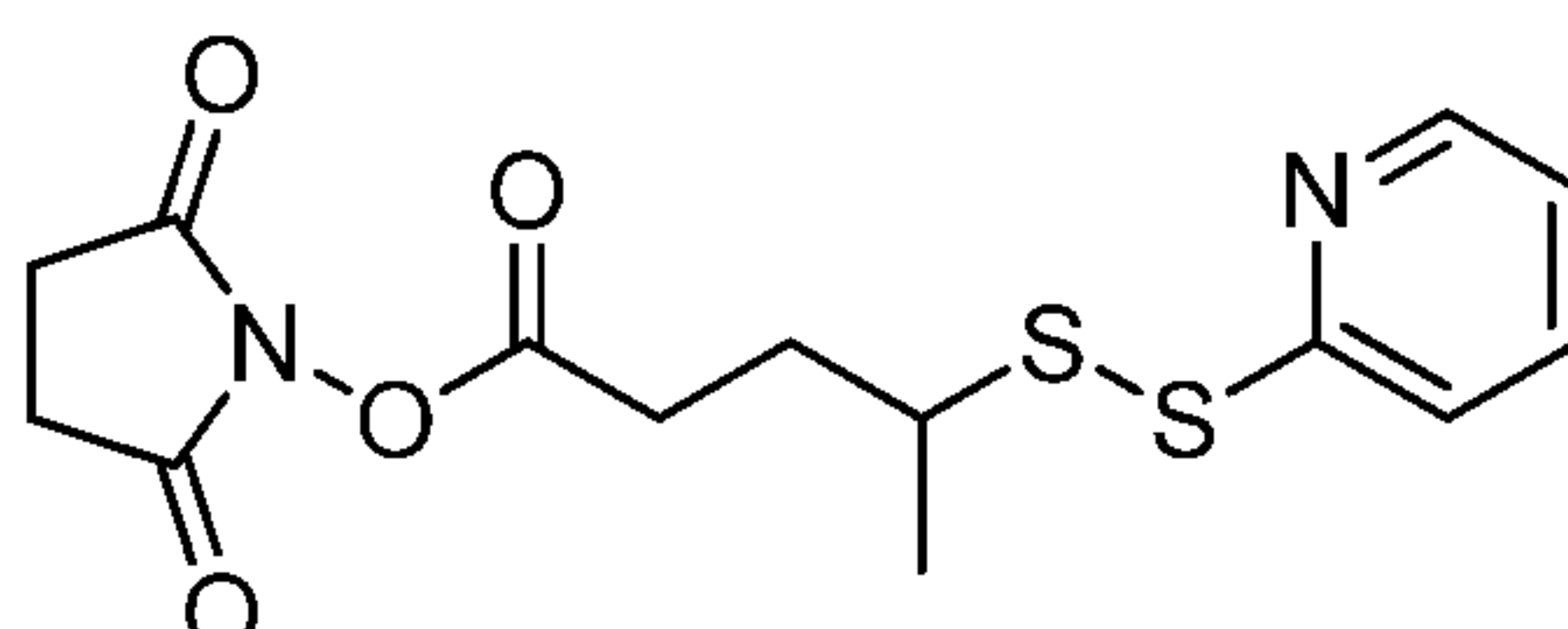


[00125] In one embodiment, the linker L is derived from N-succinimidyl iodoacetate (SIA) or N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB).

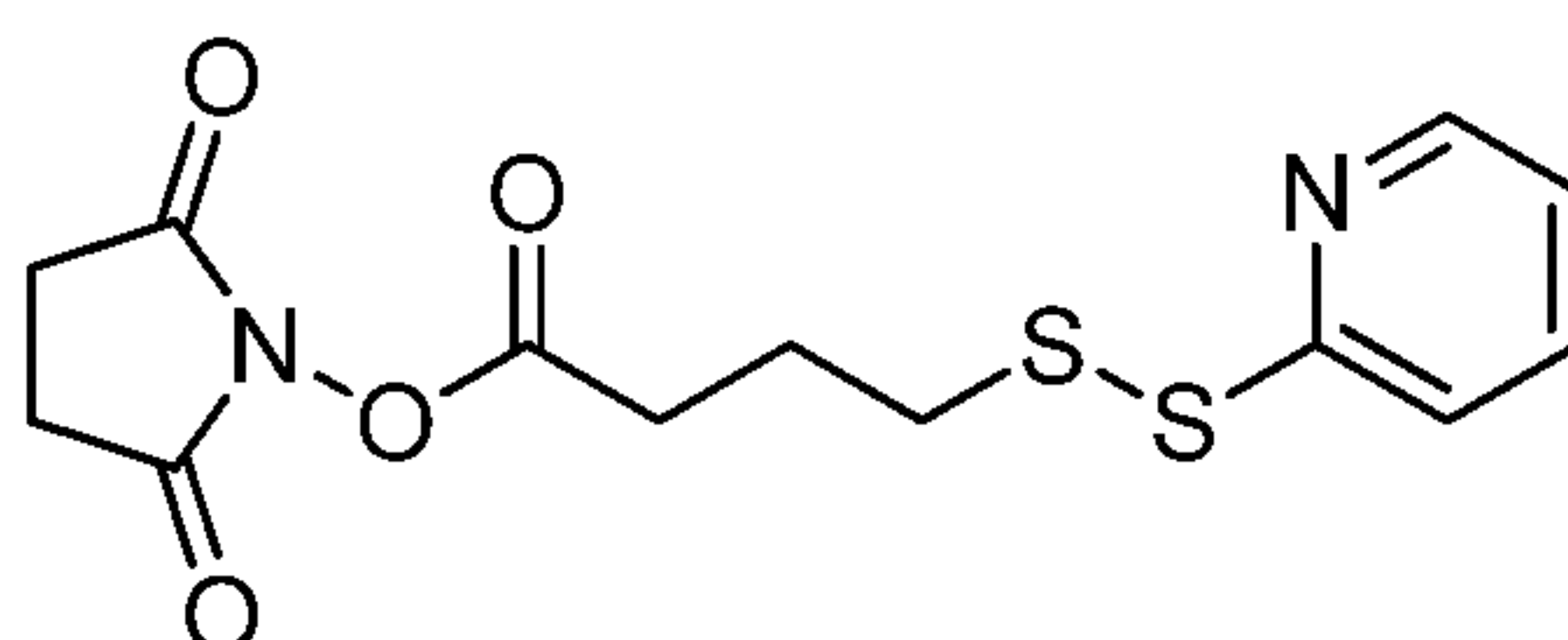
[00126] Suitable cross-linking reagents that form a cleavable linker between the drug moiety, for example maytansinoid, and the antibody are well known in the art. Disulfide containing linkers are linkers cleavable through disulfide exchange, which can occur under physiological conditions. According to the present invention, such cleavable linkers are said to be derived from disulfide-based moieties. Suitable disulfide cross-linking reagents include N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl-4-(2-pyridyldithio)butanoate (SPDB) and *N*-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), the structures of which are shown below. These disulfide cross-linking reagents form a cleavable linker derived from disulfide-based moieties.



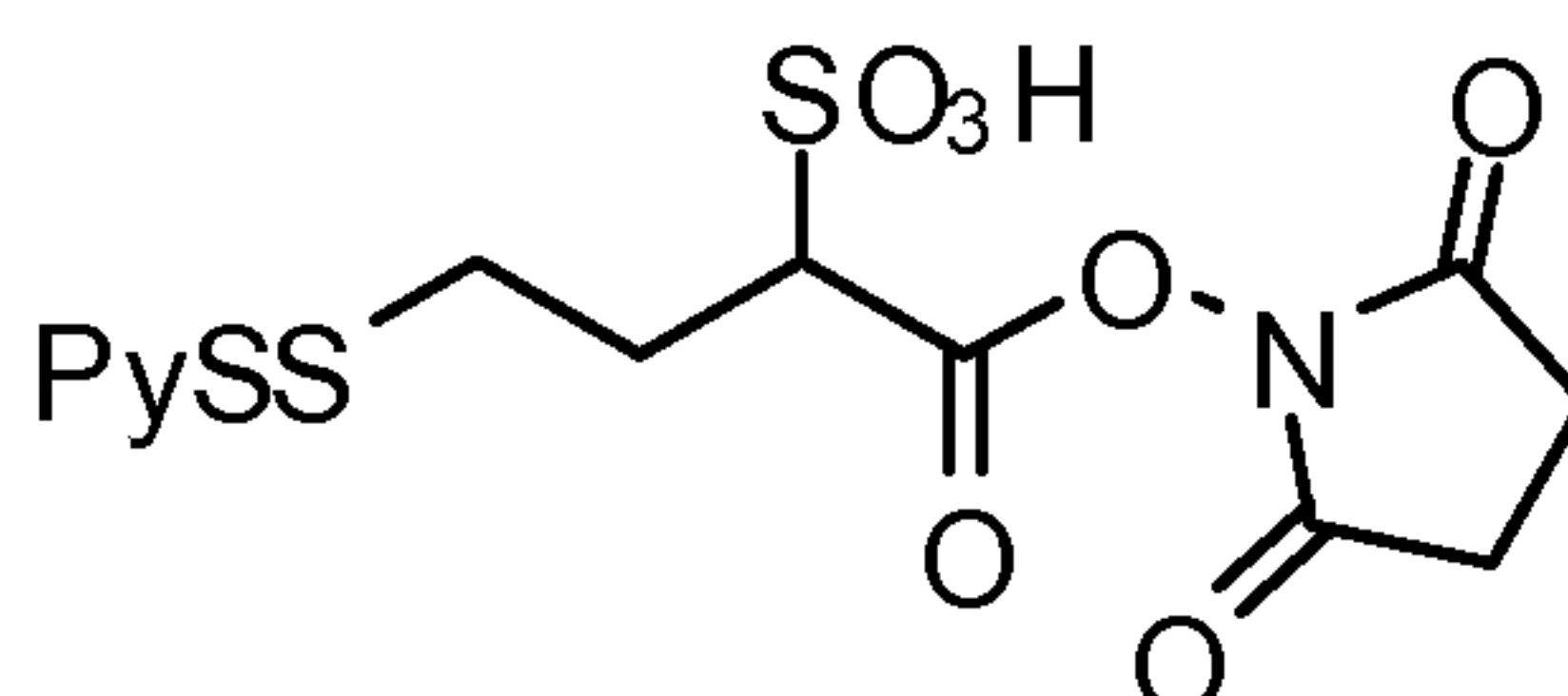
N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP),



N-succinimidyl-4-(2-pyridyldithio)pentanoate (SPP),



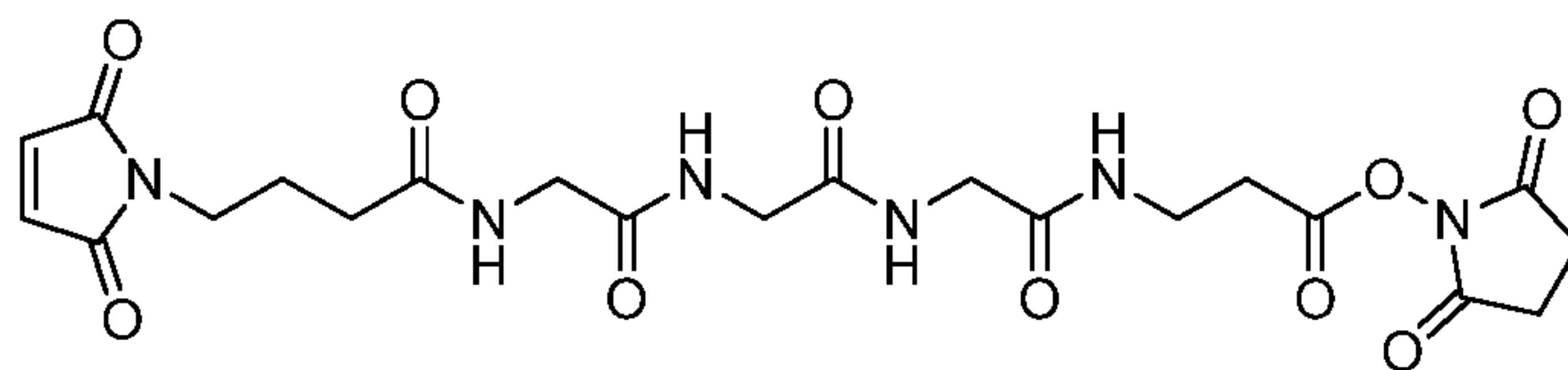
N-succinimidyl-4-(2-pyridyldithio)butanoate (SPDB) and



N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB).

[00127] In one embodiment, the linker L is derived from N-succinimidyl-4-(2-pyridyldithio) butanoate (SPDB).

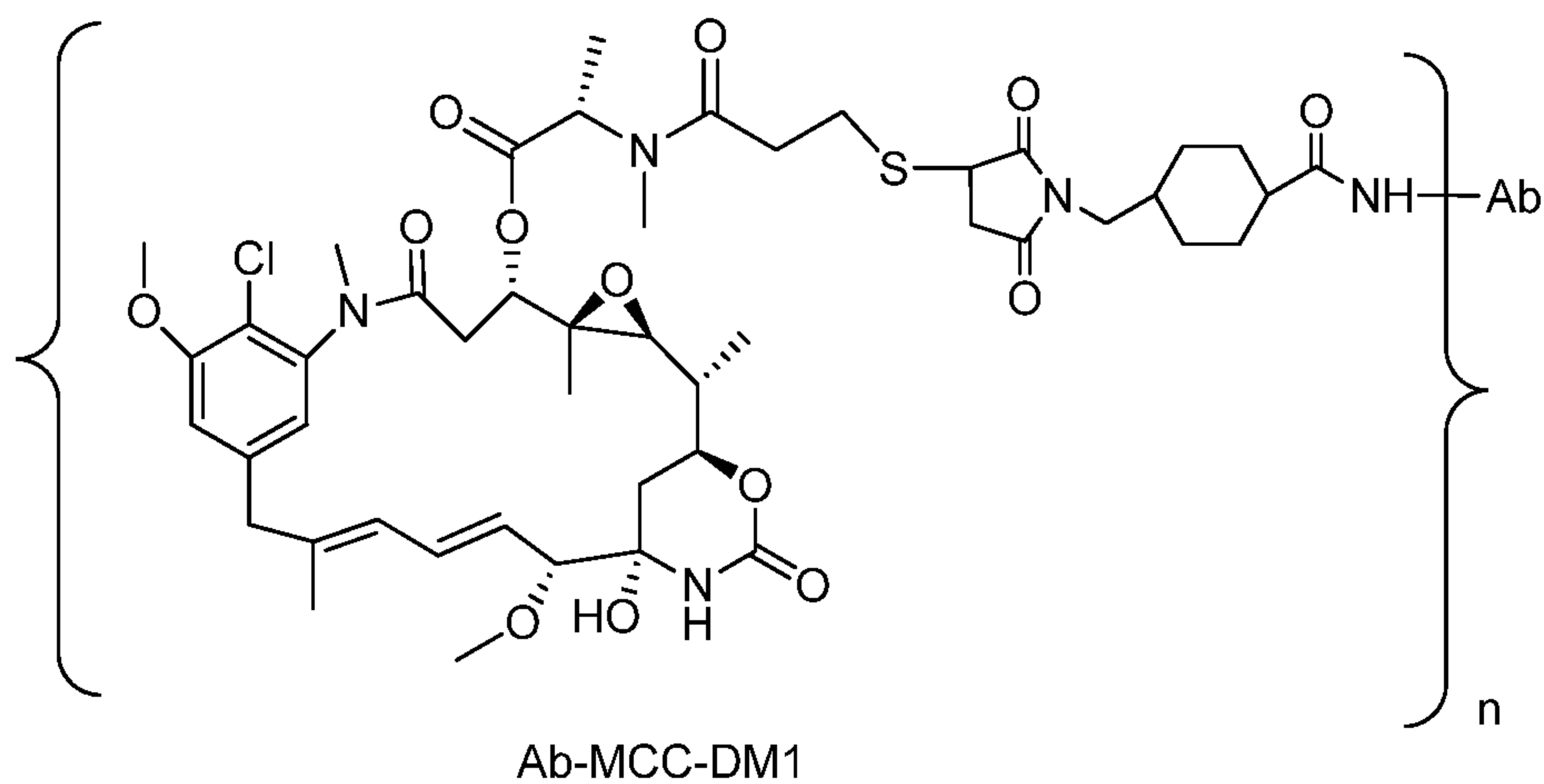
[00128] Suitable cross-linking reagents that form a charged linker between the drug moiety, for example maytansinoid, and the antibody are known as procharged cross-linking reagents. In one embodiment, the linker L is derived from the procharged cross-linking reagent CX1-1. The structure of CX1-1 is below.

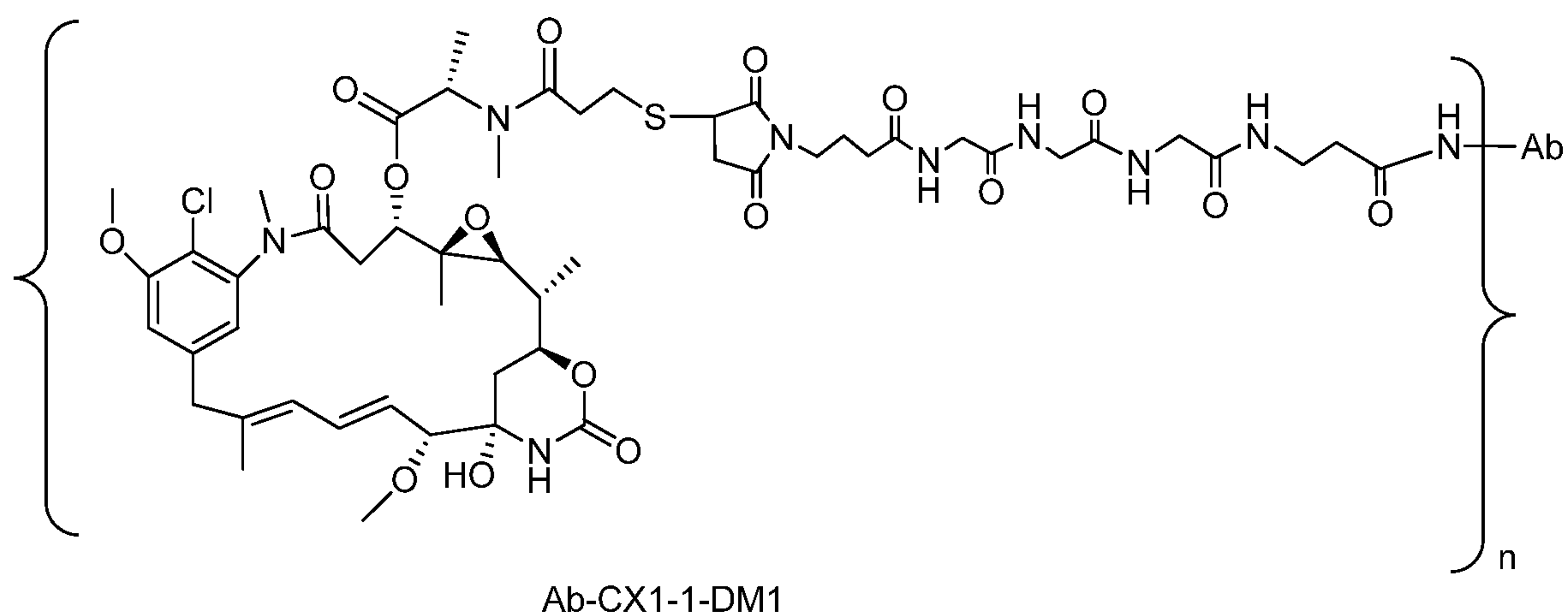
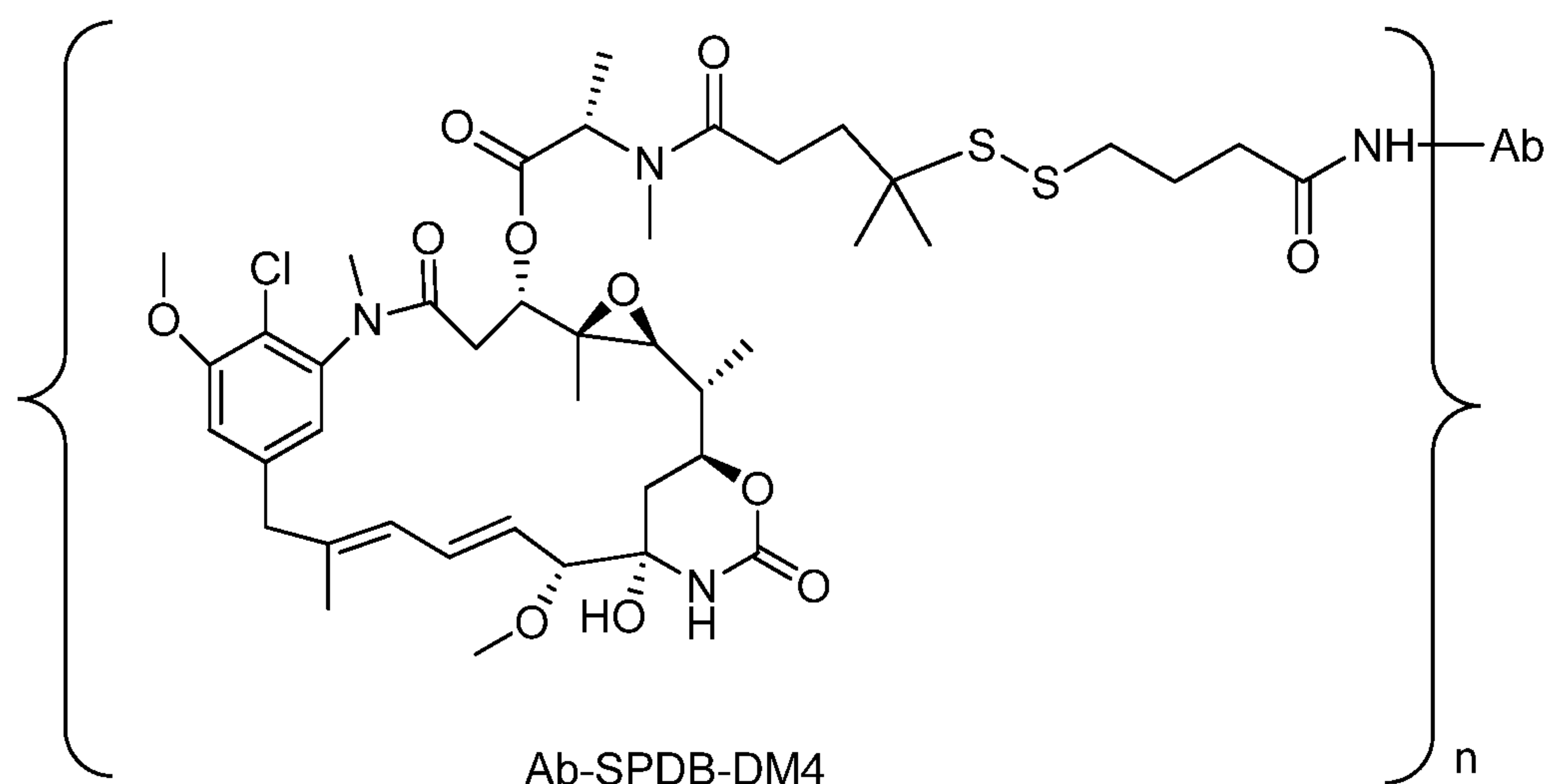


2,5-dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1)

[00129] Each of the cross-linking reagents depicted above contains, at one end of the cross-linking reagent, a NHS-ester which reacts with a primary amine of the antibody to form an amide bond and, at the other end, a maleimide group or pyridinyldisulfide group which reacts with the sulfhydryl of the maytansinoid drug moiety to form a thioether or disulfide bond.

[00130] In one embodiment, the conjugate of the present invention is represented by any one of the following structural formulae.





[00131] wherein:

[00132] Ab is an antibody or antigen binding fragment thereof that specifically binds to both human FGFR2 and FGFR4;

[00133] n, which indicates the number of D-L groups attached the Ab through the formation of an amide bond with a primary amine of the Ab, is an integer from 1 to 20. In one embodiment, n is an integer from 1 to 10, 2 to 8 or 2 to 5. In a specific embodiment, n is 3 or 4.

[00134] In one embodiment, the average molar ratio of drug (*e.g.*, DM1 or DM4) to the antibody in the conjugate (*i.e.*, average n value, also known as Maytanisnoid Antibody Ratio (MAR)) is about 1 to about 10, about 2 to about 8 (*e.g.*, 1.9, 2.0, 2.1, 2.2, 2.3, 2.4, 2.5, 2.6, 2.7, 2.8, 2.9, 3.0, 3.1, 3.2, 3.3, 3.4, 3.5, 3.6, 3.7, 3.8, 3.9, 4.0, 4.1, 4.2, 4.3, 4.4, 4.5, 4.6, 4.7, 4.8, 4.9, 5.0, 5.1, 5.2, 5.3, 5.4, 5.5, 5.6, 5.7, 5.8, 5.9, 6.0, 6.1, 6.2, 6.3, 6.4, 6.5, 6.6, 6.7, 6.8, 6.9, 7.0, 7.1,

7.2, 7.3, 7.4, 7.5, 7.6, 7.7, 7.8, 7.9, 8.0, or 8.1), about 2.5 to about 7, about 3 to about 5, about 2.5 to about 4.5 (*e.g.*, about 2.5, about 2.6, about 2.7, about 2.8, about 2.9, about 3.0, about 3.1, about 3.3, about 3.4, about 3.5, about 3.6, about 3.7, about 3.8, about 3.9, about 4.0, about 4.1, about 4.2, about 4.3, about 4.4, about 4.5), about 3.0 to about 4.0, about 3.2 to about 4.2, or about 4.5 to 5.5 (*e.g.*, about 4.5, about 4.6, about 4.7, about 4.8, about 4.9, about 5.0, about 5.1, about 5.2, about 5.3, about 5.4, or about 5.5).

[00135] In one aspect of the invention, the conjugate of the present invention has substantially high purity and has one or more of the following features: (a) greater than about 90% (*e.g.*, greater than or equal to about 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%), preferably greater than about 95%, of conjugate species are monomeric, (b) unconjugated linker level in the conjugate preparation is less than about 10% (*e.g.*, less than or equal to about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0%) (relative to total linker), (c) less than 10% of conjugate species are crosslinked (*e.g.*, less than or equal to about 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or 0%), (d) free drug (*e.g.*, DM1 or DM4) level in the conjugate preparation is less than about 2% (*e.g.*, less than or equal to about 1.5%, 1.4%, 1.3%, 1.2%, 1.1%, 1.0%, 0.9%, 0.8%, 0.7%, 0.6%, 0.5%, 0.4%, 0.3%, 0.2%, 0.1%, or 0%) (mol/mol relative to total cytotoxic agent) and/or (e) no substantial increase in the level of free drug (*e.g.*, DM1 or DM4) occurs upon storage (*e.g.*, after about 1 week, about 2 weeks, about 3 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 1 year, about 2 years, about 3 years, about 4 years, or about 5 years). “Substantial increase” in the level of free drug (*e.g.*, DM1 or DM4) means that after certain storage time (*e.g.*, about 1 week, about 2 weeks, about 3 weeks, about 1 month, about 2 months, about 3 months, about 4 months, about 5 months, about 6 months, about 1 year, about 2 years, about 3 years, about 4 years, or about 5 years), the increase in the level of free drug (*e.g.*, DM1 or DM4) is less than about 0.1%, about 0.2%, about 0.3%, about 0.4%, about 0.5%, about 0.6%, about 0.7%, about 0.8%, about 0.9%, about 1.0%, about 1.1%, about 1.2%, about 1.3%, about 1.4%, about 1.5%, about 1.6%, about 1.7%, about 1.8%, about 1.9%, about 2.0%, about 2.2%, about 2.5%, about 2.7%, about 3.0%, about 3.2%, about 3.5%, about 3.7%, or about 4.0%.

[00136] As used herein, the term “unconjugated linker” refers to the antibody that is covalently linked with a linker derived from a cross-linking reagent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1), wherein the antibody is not covalently coupled to the drug (*e.g.*,

DM1 or DM4) through a linker (i.e., the “unconjugated linker” can be represented by Ab-MCC, Ab-SPDB, or Ab-CX1-1).

1. Drug Moiety

[00137] The present invention provides immunoconjugates that specifically bind to FGFR2/4. The immunoconjugates of the invention comprise anti-FGFR2/4 antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents that are conjugated to a drug moiety, *e.g.*, an anti-cancer agent, an autoimmune treatment agent, an anti-inflammatory agent, an antifungal agent, an antibacterial agent, an anti-parasitic agent, an anti-viral agent, or an anesthetic agent. The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the invention can be conjugated to several identical or different drug moieties using any methods known in the art.

[00138] In certain embodiments, the drug moiety of the immunoconjugates of the present invention is selected from a group consisting of a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, an inhibitor of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, an RNA polymerase inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

[00139] In one embodiment, the drug moiety of the immunoconjugates of the present invention is a maytansinoid drug moiety, such as but not limited to, DM1, DM3, or DM4.

[00140] Further, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present invention may be conjugated to a drug moiety that modifies a given biological response. Drug moieties are not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein, peptide, or polypeptide possessing a desired biological activity. Such proteins may include, for example, a toxin such as abrin, ricin A, pseudomonas exotoxin, cholera toxin, or diphtheria toxin, a protein such as tumor necrosis factor, α -interferon, β -interferon, nerve growth factor, platelet derived growth factor,

tissue plasminogen activator, a cytokine, an apoptotic agent, an anti-angiogenic agent, or, a biological response modifier such as, for example, a lymphokine.

[00141] In one embodiment, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present invention are conjugated to a drug moiety, such as a cytotoxin, a drug (*e.g.*, an immunosuppressant) or a radiotoxin. Examples of cytotoxins include but are not limited to, taxanes (see, *e.g.*, International (PCT) Patent Application Nos. WO 01/38318 and PCT/US03/02675), DNA-alkylating agents (*e.g.*, CC-1065 analogs), anthracyclines, tubulysin analogs, duocarmycin analogs, auristatin E, auristatin F, maytansinoids, and cytotoxic agents comprising a reactive polyethylene glycol moiety (see, *e.g.*, Sasse *et al.*, J. Antibiot. (Tokyo), 53, 879-85 (2000), Suzawa *et al.*, Bioorg. Med. Chem., 8, 2175-84 (2000), Ichimura *et al.*, J. Antibiot. (Tokyo), 44, 1045-53 (1991), Francisco *et al.*, Blood (2003) (electronic publication prior to print publication), U.S. Pat. Nos. 5,475,092, 6,340,701, 6,372,738, and 6,436,931, U.S. Patent Application Publication No. 2001/0036923 A1, Pending U.S. patent application Ser. Nos. 10/024,290 and 10/116,053, and International (PCT) Patent Application No. WO 01/49698), taxon, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, t. colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof. Therapeutic agents also include, for example, anti-metabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), ablating agents (*e.g.*, mechlorethamine, thiotepa chlorambucil, meiphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin, anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine and vinblastine). (See *e.g.*, Seattle Genetics US20090304721).

[00142] Other examples of cytotoxins that can be conjugated to the antibodies, antibody fragments (antigen binding fragments) or functional equivalents of the invention include duocarmycins, calicheamicins, maytansines and auristatins, and derivatives thereof.

[00143] Various types of cytotoxins, linkers and methods for conjugating therapeutic agents to antibodies are known in the art, see, *e.g.*, Saito *et al.*, (2003) Adv. Drug Deliv. Rev.

55:199-215; Trail *et al.*, (2003) Cancer Immunol. Immunother. 52:328-337; Payne, (2003) Cancer Cell 3:207-212; Allen, (2002) Nat. Rev. Cancer 2:750-763; Pastan and Kreitman, (2002) Curr. Opin. Investig. Drugs 3:1089-1091; Senter and Springer, (2001) Adv. Drug Deliv. Rev. 53:247-264.

[00144] The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present invention can also be conjugated to a radioactive isotope to generate cytotoxic radiopharmaceuticals, referred to as radioimmunoconjugates. Examples of radioactive isotopes that can be conjugated to antibodies for use diagnostically or therapeutically include, but are not limited to, iodine-131, indium-111, yttrium-90, and lutetium-177. Methods for preparing radioimmunoconjugates are established in the art. Examples of radioimmunoconjugates are commercially available, including Zevalin® (DEC Pharmaceuticals) and Bexxar® (Corixa Pharmaceuticals), and similar methods can be used to prepare radioimmunoconjugates using the antibodies of the invention. In certain embodiments, the macrocyclic chelator is 1,4,7,10-tetraazacyclododecane-N,N',N'',N'''-tetraacetic acid (DOTA) which can be attached to the antibody via a linker molecule. Such linker molecules are commonly known in the art and described in Denardo *et al.*, (1998) Clin Cancer Res. 4(10):2483-90; Peterson *et al.*, (1999) Bioconjug. Chem. 10(4):553-7; and Zimmerman *et al.*, (1999) Nucl. Med. Biol. 26(8):943-50, each incorporated by reference in their entireties.

[00145] The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present invention can also be conjugated to a heterologous protein or polypeptide (or fragment thereof, preferably to a polypeptide of at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90 or at least 100 amino acids) to generate fusion proteins. In particular, the invention provides fusion proteins comprising an antibody fragment (*e.g.*, antigen binding fragment) described herein (*e.g.*, a Fab fragment, Fd fragment, Fv fragment, F(ab)₂ fragment, a VH domain, a VH CDR, a VL domain or a VL CDR) and a heterologous protein, polypeptide, or peptide.

[00146] Additional fusion proteins may be generated through the techniques of gene-shuffling, motif-shuffling, exon-shuffling, and/or codon-shuffling (collectively referred to as “DNA shuffling”). DNA shuffling may be employed to alter the activities of antibodies of the invention or fragments thereof (*e.g.*, antibodies or fragments thereof with higher affinities and lower dissociation rates). See, generally, U.S. Patent Nos. 5,605,793, 5,811,238, 5,830,721,

5,834,252, and 5,837,458; Patten *et al.*, (1997) *Curr. Opin. Biotechnol.* 8:724-33; Harayama, (1998) *Trends Biotechnol.* 16(2):76-82; Hansson *et al.*, (1999) *J. Mol. Biol.* 287:265-76; and Lorenzo and Blasco, (1998) *Biotechniques* 24(2):308- 313 (each of these patents and publications are hereby incorporated by reference in its entirety). Antibodies or fragments thereof, or the encoded antibodies or fragments thereof, may be altered by being subjected to random mutagenesis by error-prone PCR, random nucleotide insertion or other methods prior to recombination. A polynucleotide encoding an antibody or fragment thereof that specifically binds to an antigen may be recombined with one or more components, motifs, sections, parts, domains, fragments, etc. of one or more heterologous molecules.

[00147] Moreover, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present invention can be conjugated to marker sequences, such as a peptide, to facilitate purification. In preferred embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN®, Inc., 9259 Eton Avenue, Chatsworth, CA, 91311), among others, many of which are commercially available. As described in Gentz *et al.*, (1989) *Proc. Natl. Acad. Sci. USA* 86:821-824, for instance, hexa-histidine provides for convenient purification of the fusion protein. Other peptide tags useful for purification include, but are not limited to, the hemagglutinin (“HA”) tag, which corresponds to an epitope derived from the influenza hemagglutinin protein (Wilson *et al.*, (1984) *Cell* 37:767), and the “FLAG®” tag (A. Einhauer *et al.*, *J. Biochem. Biophys. Methods* 49: 455–465, 2001). According to the present invention, antibodies or antigen binding fragments can also be conjugated to tumor-penetrating peptides in order to enhance their efficacy.

[00148] In other embodiments, the antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the present invention are conjugated to a diagnostic or detectable agent. Such immunoconjugates can be useful for monitoring or prognosing the onset, development, progression and/or severity of a disease or disorder as part of a clinical testing procedure, such as determining the efficacy of a particular therapy. Such diagnosis and detection can be accomplished by coupling the antibody to detectable substances including, but not limited to, various enzymes, such as, but not limited to, horseradish peroxidase, alkaline phosphatase, beta-galactosidase, or acetylcholinesterase; prosthetic groups, such as, but not limited to, streptavidin/biotin and avidin/biotin; fluorescent materials, such as, but not limited to, Alexa Fluor® 350, Alexa Fluor® 405, Alexa Fluor® 430, Alexa Fluor® 488, Alexa Fluor® 500, Alexa

Fluor® 514, Alexa Fluor® 532, Alexa Fluor® 546, Alexa Fluor® 555, Alexa Fluor® 568, Alexa Fluor® 594, Alexa Fluor® 610, Alexa Fluor® 633, Alexa Fluor® 647, Alexa Fluor® 660, Alexa Fluor® 680, Alexa Fluor® 700, Alexa Fluor® 750, umbelliferone, fluorescein, fluorescein isothiocyanate, rhodamine, dichlorotriazinylamine fluorescein, dansyl chloride or phycoerythrin; luminescent materials, such as, but not limited to, luminol; bioluminescent materials, such as but not limited to, luciferase, luciferin, and aequorin; radioactive materials, such as, but not limited to, iodine (^{131}I , ^{125}I , ^{123}I , and ^{121}I), carbon (^{14}C), sulfur (^{35}S), tritium (^3H), indium (^{115}In , ^{113}In , ^{112}In , and ^{111}In), technetium (^{99}Tc), thallium (^{201}Tl), gallium (^{68}Ga , ^{67}Ga), palladium (^{103}Pd), molybdenum (^{99}Mo), xenon (^{133}Xe), fluorine (^{18}F), ^{153}Sm , ^{177}Lu , ^{159}Gd , ^{149}Pm , ^{140}La , ^{175}Yb , ^{166}Ho , ^{90}Y , ^{47}Sc , ^{186}Re , ^{188}Re , ^{142}Pr , ^{105}Rh , ^{97}Ru , ^{68}Ge , ^{57}Co , ^{65}Zn , ^{85}Sr , ^{32}P , ^{153}Gd , ^{169}Yb , ^{51}Cr , ^{54}Mn , ^{75}Se , ^{64}Cu , ^{113}Sn , and ^{117}Sn ; and positron emitting metals using various positron emission tomographies, and non-radioactive paramagnetic metal ions.

[00149] The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or functional equivalents of the invention may also be attached to solid supports, which are particularly useful for immunoassays or purification of the target antigen. Such solid supports include, but are not limited to, glass, cellulose, polyacrylamide, nylon, polystyrene, polyvinyl chloride or polypropylene.

2. Linker

[00150] As used herein, a "linker" is any chemical moiety that is capable of linking an antibody, antibody fragment (*e.g.*, antigen binding fragments) or functional equivalent to another moiety, such as a drug moiety. In some instances, part of the linker is provided by the maytansinoid. For example, DM1, a thiol containing maytansinoid, is a derivative of the natural maytansinoid, and provides part of the linker. The side chain at the C-3 hydroxyl group of the maytansine ends in CO-CH_3 , the side chain of DM1 ends in $\text{CO-CH}_2\text{-CH}_2\text{-SH}$. Therefore the final linker is assembled from two pieces, the cross-linking reagent introduced into the antibody and the side chain from DM1. Linkers can be susceptible to cleavage (cleavable linker), such as, acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage, and disulfide bond cleavage, at conditions under which the compound or the antibody remains active. Alternatively, linkers can be substantially resistant to cleavage (*e.g.*, stable linker or noncleavable linker). In some aspects, the linker is a procharged linker, a hydrophilic linker, or a dicarboxylic acid based linker.

[00151] Non-cleavable linkers are any chemical moiety capable of linking a drug, such as a maytansinoid, to an antibody in a stable, covalent manner and does not fall off under the categories listed above for cleavable linkers. Thus, non-cleavable linkers are substantially resistant to acid-induced cleavage, photo-induced cleavage, peptidase-induced cleavage, esterase-induced cleavage and disulfide bond cleavage. Furthermore, non-cleavable refers to the ability of the chemical bond in the linker or adjoining to the linker to withstand cleavage induced by an acid, photolabile-cleaving agent, a peptidase, an esterase, or a chemical or physiological compound that cleaves a disulfide bond, at conditions under which the drug, such as maytansinoid or the antibody does not lose its activity.

[00152] Disulfide containing linkers are linkers cleavable through disulfide exchange, which can occur under physiological conditions. Acid-labile linkers are linkers cleavable at acidic pH. For example, certain intracellular compartments, such as endosomes and lysosomes, have an acidic pH (pH 4-5), and provide conditions suitable to cleave acid-labile linkers.

[00153] Photo-labile linkers are linkers that are useful at the body surface and in many body cavities that are accessible to light. Furthermore, infrared light can penetrate tissue.

[00154] Some linkers can be cleaved by peptidases, i.e. peptidase cleavable linkers. Only certain peptides are readily cleaved inside or outside cells, see e.g. Trout et al., 79 Proc. Natl. Acad.Sci. USA, 626-629 (1982) and Umemoto et al. 43 Int. J. Cancer, 677-684 (1989). Furthermore, peptides are composed of α -amino acids and peptidic bonds, which chemically are amide bonds between the carboxylate of one amino acid and the amino group of a second amino acid. Other amide bonds, such as the bond between a carboxylate and the ϵ -amino group of lysine, are understood not to be peptidic bonds and are considered non-cleavable.

[00155] Some linkers can be cleaved by esterases, i.e. esterase cleavable linkers. Again, only certain esters can be cleaved by esterases present inside or outside of cells. Esters are formed by the condensation of a carboxylic acid and an alcohol. Simple esters are esters produced with simple alcohols, such as aliphatic alcohols, and small cyclic and small aromatic alcohols.

[00156] Procharged linkers are derived from charged cross-linking reagents that retain their charge after incorporation into an antibody drug conjugate. Examples of procharged linkers can be found in US 2009/0274713.

[00157] In one aspect, the linker used in the present invention is derived from a crosslinking reagent such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-

succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC) or 2,5-dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1). In another aspect, the linker used in the present invention is derived from a cross-linking agent such as N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC), N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB) or 2,5-dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1).

3. Conjugation and Preparation of ADCs

[00158] The conjugates of the present invention can be prepared by any methods known in the art, such as those described in US Patent Nos. 7,811,572, 6,411,163, 7,368,565, and 8,163,888, and US application publications 2011/0003969, 2011/0166319, 2012/0253021 and 2012/0259100. The entire teachings of these patents and patent application publications are herein incorporated by reference.

One-Step Process

[00159] In one embodiment, the conjugates of the present invention can be prepared by a one-step process. The process comprises combining the antibody, drug and cross-linking agent in a substantially aqueous medium, optionally containing one or more co-solvents, at a suitable pH. In one embodiment, the process comprises the step of contacting the antibody of the present invention with a drug (*e.g.*, DM1 or DM4) to form a first mixture comprising the antibody and the drug, and then contacting the first mixture comprising the antibody and the drug with a cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate (*e.g.*, Ab-MCC-DM1, Ab-SPDB-DM4, or Ab-CX1-1-DM1), (ii) free drug (*e.g.*, DM1 or DM4), and (iii) reaction by-products.

[00160] In one embodiment, the one-step process comprises contacting the antibody with the drug (*e.g.*, DM1 or DM4) and then the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) in a solution having a pH of about 6 or greater (*e.g.*, about 6 to about 9,

about 6 to about 7, about 7 to about 9, about 7 to about 8.5, about 7.5 to about 8.5, about 7.5 to about 8.0, about 8.0 to about 9.0, or about 8.5 to about 9.0). For example, the inventive process comprises contacting a cell-binding agent with the drug (DM1 or DM4) and then the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) in a solution having a pH of about 6.0, about 6.1, about 6.2, about 6.3, about 6.4, about 6.5, about 6.6, about 6.7, about 6.8, about 6.9, about 7.0, about 7.1, about 7.2, about 7.3, about 7.4, about 7.5, about 7.6, about 7.7, about 7.8, about 7.9, about 8.0, about 8.1, about 8.2, about 8.3, about 8.4, about 8.5, about 8.6, about 8.7, about 8.8, about 8.9, or about 9.0. In a specific embodiment, the inventive process comprises contacting a cell-binding agent with the drug (*e.g.*, DM1 or DM4) and then the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) in a solution having a pH of about 7.8 (*e.g.*, a pH of 7.6 to 8.0 or a pH of 7.7 to 7.9).

[00161] The one-step process (*i.e.*, contacting the antibody with the drug (*e.g.*, DM1 or DM4) and then the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) can be carried out at any suitable temperature known in the art. For example, the one-step process can occur at about 20°C or less (*e.g.*, about -10°C (provided that the solution is prevented from freezing, *e.g.*, by the presence of organic solvent used to dissolve the cytotoxic agent and the bifunctional crosslinking reagent) to about 20°C, about 0°C to about 18°C, about 4°C to about 16°C), at room temperature (*e.g.*, about 20°C to about 30°C or about 20°C to about 25°C), or at an elevated temperature (*e.g.*, about 30°C to about 37°C). In one embodiment, the one-step process occurs at a temperature of about 16°C to about 24°C (*e.g.*, about 16°C, about 17°C, about 18°C, about 19°C, about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, or about 25°C). In another embodiment, the one-step process is carried out at a temperature of about 15°C or less (*e.g.*, about -10°C to about 15°C, or about 0°C to about 15°C). For example, the process comprises contacting the antibody with the drug (*e.g.*, DM1 or DM4) and then the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) at a temperature of about 15°C, about 14°C, about 13°C, about 12°C, about 11°C, about 10°C, about 9°C, about 8°C, about 7°C, about 6°C, about 5°C, about 4°C, about 3°C, about 2°C, about 1°C, about 0°C, about -1°C, about -2°C, about -3°C, about -4°C, about -5°C, about -6°C, about -7°C, about -8°C, about -9°C, or about -10°C, provided that the solution is prevented from freezing, *e.g.*, by the presence of organic solvent(s) used to dissolve the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, Sulfo-SPDB SPDB, or CX1-1). In one embodiment, the process comprises contacting the antibody with the drug (*e.g.*, DM1 or DM4) and then the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) at a temperature of about -10°C to about 15°C, about 0°C to about 15°C, about 0°C to about 10°C, about 0°C to about 5°C, about 5°C to about 15°C, about 10°C to about

15°C, or about 5°C to about 10°C. In another embodiment, the process comprises contacting the antibody with the drug (*e.g.*, DM1 or DM4) and then the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) at a temperature of about 10°C (*e.g.*, a temperature of 8°C to 12°C or a temperature of 9°C to 11°C).

[00162] In one embodiment, the contacting described above is effected by providing the antibody, then contacting the antibody with the drug (*e.g.*, DM1 or DM4) to form a first mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4), and then contacting the first mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). For example, in one embodiment, the antibody is provided in a reaction vessel, the drug (*e.g.*, DM1 or DM4) is added to the reaction vessel (thereby contacting the antibody), and then the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) is added to the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) (thereby contacting the mixture comprising the antibody and the drug). In one embodiment, the antibody is provided in a reaction vessel, and the drug (*e.g.*, DM1 or DM4) is added to the reaction vessel immediately following providing the antibody to the vessel. In another embodiment, the antibody is provided in a reaction vessel, and the drug (*e.g.*, DM1 or DM4) is added to the reaction vessel after a time interval following providing the antibody to the vessel (*e.g.*, about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 40 minutes, about 50 minutes, about 1 hour, about 1 day or longer after providing the cell-binding agent to the space). The drug (*e.g.*, DM1 or DM4) can be added quickly (*i.e.*, within a short time interval, such as about 5 minutes, about 10 minutes) or slowly (such as by using a pump).

[00163] The mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) can then be contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) either immediately after contacting the antibody with the drug (*e.g.*, DM1 or DM4) or at some later point (*e.g.*, about 5 minutes to about 8 hours or longer) after contacting the antibody with the drug (*e.g.*, DM1 or DM4). For example, in one embodiment, the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) is added to the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) immediately after the addition of the drug (*e.g.*, DM1 or DM4) to the reaction vessel comprising the antibody. Alternatively, the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) can be contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) at about 5 minutes, about 10 minutes, about 20 minutes, about 30 minutes, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, or longer after contacting the antibody with the drug (*e.g.*, DM1 or DM4).

[00164] After the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) is contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) the reaction is allowed to proceed for about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, or longer (*e.g.*, about 30 hours, about 35 hours, about 40 hours, about 45 hours, or about 48 hrs).

[00165] In one embodiment, the one-step process further comprises a quenching step to quench any unreacted drug (*e.g.*, DM1 or DM4) and/or unreacted cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). The quenching step is typically performed prior to purification of the conjugate. In one embodiment, the mixture is quenched by contacting the mixture with a quenching reagent. As used herein, the “quenching reagent” refers to a reagent that reacts with the free drug (*e.g.*, DM1 or DM4) and/or cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). In one embodiment, maleimide or haloacetamide quenching reagents, such as 4-maleimidobutyric acid, 3-maleimidopropionic acid, N-ethylmaleimide, iodoacetamide, or iodoacetamidopropionic acid, can be used to ensure that any unreacted group (such as thiol) in the drug (*e.g.*, DM1 or DM4) is quenched. The quenching step can help prevent the dimerization of the drug (*e.g.*, DM1). The dimerized DM1 can be difficult to remove. Upon quenching with polar, charged thiol-quenching reagents (such as 4-maleimidobutyric acid or 3-maleimidopropionic acid), the excess, unreacted DM1 is converted into a polar, charged, water-soluble adduct that can be easily separated from the covalently-linked conjugate during the purification step. Quenching with non-polar and neutral thiol-quenching reagents can also be used. In one embodiment, the mixture is quenched by contacting the mixture with a quenching reagent that reacts with the unreacted cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). For example, nucleophiles can be added to the mixture in order to quench any unreacted SMCC. The nucleophile preferably is an amino group containing nucleophile, such as lysine, taurine and hydroxylamine.

[00166] In a preferred embodiment, the reaction (*i.e.*, contacting the antibody with the drug (*e.g.*, DM1 or DM4) and then cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1)) is allowed to proceed to completion prior to contacting the mixture with a quenching reagent. In this regard, the quenching reagent is added to the mixture about 1 hour to about 48 hours (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12

hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, or about 25 hours to about 48 hours) after the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) is contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1).

[00167] Alternatively, the mixture is quenched by lowering the pH of the mixture to about 5.0 (*e.g.*, 4.8, 4.9, 5.0, 5.1 or 5.2). In another embodiment, the mixture is quenched by lowering the pH to less than 6.0, less than 5.5, less than 5.0, less than 4.8, less than 4.6, less than 4.4, less than 4.2, less than 4.0. Alternatively, the pH is lowered to about 4.0 (*e.g.*, 3.8, 3.9, 4.0, 4.1 or 4.2) to about 6.0 (*e.g.*, 5.8, 5.9, 6.0, 6.1 or 6.2), about 4.0 to about 5.0, about 4.5 (*e.g.*, 4.3, 4.4, 4.5, 4.6 or 4.7) to about 5.0. In one embodiment, the mixture is quenched by lowering the pH of the mixture to 4.8. In another embodiment, the mixture is quenched by lowering the pH of the mixture to 5.5.

[00168] In one embodiment, the one-step process further comprises a holding step to release the unstably bound linkers from the antibody. The holding step comprises holding the mixture prior to purification of the conjugate (*e.g.*, after the reaction step, between the reaction step and the quenching step, or after the quenching step). For example, the process comprises (a) contacting the antibody with the drug (*e.g.*, DM1 or DM4) to form a mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4); and then contacting the mixture comprising the antibody and drug (*e.g.*, DM1 or DM4) with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1), in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate (*e.g.*, Ab-MCC-DM1, Ab-SPDB-DM4 or Ab-CX1-1-DM1), (ii) free drug (*e.g.*, DM1 or DM4), and (iii) reaction by-products, (b) holding the mixture prepared in step (a) to release the unstably bound linkers from the cell-binding agent, and (c) purifying the mixture to provide a purified conjugate.

[00169] In another embodiment, the process comprises (a) contacting the antibody with the drug (*e.g.*, DM1 or DM4) to form a mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4); and then contacting the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1), in a solution having a pH of about 4 to about 9 to provide a mixture comprising (i) the conjugate, (ii) free drug (*e.g.*, DM1 or DM4), and (iii) reaction by-products, (b) quenching the mixture prepared in step (a) to quench any unreacted drug (*e.g.*, DM1 or DM4) and/or unreacted cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1), (c) holding the mixture prepared in step (b) to release the unstably bound linkers from the cell-binding agent, and (d) purifying the

mixture to provide a purified conjugate (*e.g.*, Ab-MCC-DM1, Ab-SPDB-DM4 or Ab-CX1-1-DM1).

[00170] Alternatively, the holding step can be performed after purification of the conjugate, followed by an additional purification step.

[00171] In a preferred embodiment, the reaction is allowed to proceed to completion prior to the holding step. In this regard, the holding step can be performed about 1 hour to about 48 hours (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours, about 9 hours, about 10 hours, about 11 hours, about 12 hours, about 13 hours, about 14 hours, about 15 hours, about 16 hours, about 17 hours, about 18 hours, about 19 hours, about 20 hours, about 21 hours, about 22 hours, about 23 hours, about 24 hours, or about 24 hours to about 48 hours) after the mixture comprising the antibody and the drug (*e.g.*, DM1 or DM4) is contacted with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1).

[00172] The holding step comprises maintaining the solution at a suitable temperature (*e.g.*, about 0°C to about 37°C) for a suitable period of time (*e.g.*, about 1 hour to about 1 week, about 1 hour to about 24 hours, about 1 hour to about 8 hours, or about 1 hour to about 4 hours) to release the unstably bound linkers from the antibody while not substantially releasing the stably bound linkers from the antibody. In one embodiment, the holding step comprises maintaining the solution at about 20 °C or less (*e.g.*, about 0°C to about 18°C, about 4°C to about 16°C), at room temperature (*e.g.*, about 20°C to about 30°C or about 20°C to about 25°C), or at an elevated temperature (*e.g.*, about 30°C to about 37°C). In one embodiment, the holding step comprises maintaining the solution at a temperature of about 16°C to about 24°C (*e.g.*, about 15°C, about 16°C, about 17°C, about 18°C, about 19°C, about 20°C, about 21°C, about 22°C, about 23°C, about 24°C, or about 25°C). In another embodiment, the holding step comprises maintaining the solution at a temperature of about 2°C to about 8°C (*e.g.*, about 0°C, about 1°C, about 2°C, about 3°C, about 4°C, about 5°C, about 6°C, about 7°C, about 8°C, about 9°C, or about 10°C). In another embodiment, the holding step comprises maintaining the solution at a temperature of about 37°C (*e.g.*, about 34°C, about 35°C, about 36°C, about 37°C, about 38°C, about 39°C, or about 40°C).

[00173] The duration of the holding step depends on the temperature and the pH at which the holding step is performed. For example, the duration of the holding step can be substantially reduced by performing the holding step at elevated temperature, with the maximum temperature limited by the stability of the cell-binding agent-cytotoxic agent conjugate. The holding step can comprise maintaining the solution for about 1 hour to about 1 day (*e.g.*, about 1 hour, about 2 hours, about 3 hours, about 4 hours, about 5 hours, about 6 hours, about 7 hours, about 8 hours,

about 9 hours, about 10 hours, about 12 hours, about 14 hours, about 16 hours, about 18 hours, about 20 hours, about 22 hours, or about 24 hours), about 10 hours to about 24 hours, about 12 hours to about 24 hours, about 14 hours to about 24 hours, about 16 hours to about 24 hours, about 18 hours to about 24 hours, about 20 hours to about 24 hours, about 5 hours to about 1 week, about 20 hours to about 1 week, about 12 hours to about 1 week (*e.g.*, about 12 hours, about 16 hours, about 20 hours, about 24 hours, about 2 days, about 3 days, about 4 days, about 5 days, about 6 days, or about 7 days), or about 1 day to about 1 week.

[00174] In one embodiment, the holding step comprises maintaining the solution at a temperature of about 2 °C to about 8 °C for a period of at least about 12 hours for up to a week. In another embodiment, the holding step comprises maintaining the solution at a temperature of about 2 °C to about 8 °C overnight (*e.g.*, about 12 to about 24 hours, preferably about 20 hours).

[00175] The pH value for the holding step preferably is about 4 to about 10. In one embodiment, the pH value for the holding step is about 4 or more, but less than about 6 (*e.g.*, 4 to 5.9) or about 5 or more, but less than about 6 (*e.g.*, 5 to 5.9). In another embodiment, the pH values for the holding step range from about 6 to about 10 (*e.g.*, about 6.5 to about 9, about 6 to about 8). For example, pH values for the holding step can be about 6, about 6.5, about 7, about 7.5, about 8, about 8.5, about 9, about 9.5, or about 10.

[00176] In specific embodiments, the holding step can comprise incubating the mixture at 25°C at a pH of about 6-7.5 for about 12 hours to about 1 week, incubating the mixture at 4°C at a pH of about 4.5-5.9 for about 5 hours to about 5 days, or incubating the mixture at 25°C at a pH of about 4.5-5.9 for about 5 hours to about 1 day.

[00177] The one-step process may optionally include the addition of sucrose to the reaction step to increase solubility and recovery of the conjugates. Desirably, sucrose is added at a concentration of about 0.1% (w/v) to about 20% (w/v) (*e.g.*, about 0.1% (w/v), 1% (w/v), 5% (w/v), 10% (w/v), 15% (w/v), or 20% (w/v)). Preferably, sucrose is added at a concentration of about 1% (w/v) to about 10% (w/v) (*e.g.*, about 0.5% (w/v), about 1% (w/v), about 1.5% (w/v), about 2% (w/v), about 3% (w/v), about 4% (w/v), about 5% (w/v), about 6% (w/v), about 7% (w/v), about 8% (w/v), about 9% (w/v), about 10% (w/v), or about 11% (w/v)). In addition, the reaction step also can comprise the addition of a buffering agent. Any suitable buffering agent known in the art can be used. Suitable buffering agents include, for example, a citrate buffer, an acetate buffer, a succinate buffer, and a phosphate buffer. In one embodiment, the buffering agent is selected from the group consisting of HEPPSO (N-(2-hydroxyethyl)piperazine-N'-(2-hydroxypropanesulfonic acid)), POPSO (piperazine-1,4-bis-(2-hydroxy-propane-sulfonic acid) dehydrate), HEPES (4-(2-hydroxyethyl)piperazine-1-ethanesulfonic acid), HEPPS (EPPS) (4-(2-

hydroxyethyl)piperazine-1-propanesulfonic acid), TES (N-[tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid), and a combination thereof.

[00178] In one embodiment, the one-step process can further comprise the step of purifying the mixture to provide purified conjugate (*e.g.*, Ab-MCC-DM1, Ab-SPDB-DM4 or Ab-CX1-1-DM1). Any purification methods known in the art can be used to purify the conjugates of the present invention. In one embodiment, the conjugates of the present invention using tangential flow filtration (TFF), non-adsorptive chromatography, adsorptive chromatography, adsorptive filtration, selective precipitation, or any other suitable purification process, as well as combinations thereof. In another embodiment, prior to subjecting the conjugates to purification process described above, the conjugates are first filtered through one or more PVDF membranes. Alternatively, the conjugates are filtered through one or more PVDF membranes after subjecting the conjugates to the purification process described above. For example, in one embodiment, the conjugates are filtered through one or more PVDF membranes and then purified using tangential flow filtration. Alternatively, the conjugates are purified using tangential flow filtration and then filtered through one or more PVDF membranes.

[00179] Any suitable TFF systems may be utilized for purification, including a Pellicon® type system (Millipore, Billerica, MA), a Sartocan® Cassette system (Sartorius AG, Edgewood, NY), and a Centrasette™ type system (Pall Corp., East Hills, NY).

[00180] Any suitable adsorptive chromatography resin may be utilized for purification. Preferred adsorptive chromatography resins include hydroxyapatite chromatography, hydrophobic charge induction chromatography (HCIC), hydrophobic interaction chromatography (HIC), ion exchange chromatography, mixed mode ion exchange chromatography, immobilized metal affinity chromatography (IMAC), dye ligand chromatography, affinity chromatography, reversed phase chromatography, and combinations thereof. Examples of suitable hydroxyapatite resins include ceramic hydroxyapatite (CHT Type I and Type II, Bio-Rad Laboratories, Hercules, CA), HA Ultrogel® hydroxyapatite (Pall Corp., East Hills, NY), and ceramic fluoroapatite (CFT Type I and Type II, Bio-Rad Laboratories, Hercules, CA). An example of a suitable HCIC resin is MEP Hypercel™ resin (Pall Corp., East Hills, NY). Examples of suitable HIC resins include Butyl-Sepharose®, Hexyl-Sepharose®, Phenyl-Sepharose®, and Octyl Sepharose® resins (all from GE Healthcare, Piscataway, NJ), as well as Macro-prep Methyl and Macro-Prep t-Butyl resins (Biorad Laboratories, Hercules, CA). Examples of suitable ion exchange resins include SP-Sepharose®, CM-Sepharose®, and Q-Sepharose® resins (all from GE Healthcare, Piscataway, NJ), and Unosphere™ S resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable mixed mode ion exchangers include Bakerbond® ABx resin (JT Baker, Phillipsburg NJ). Examples of

suitable IMAC resins include Chelating Sepharose® resin (GE Healthcare, Piscataway, NJ) and Profinity IMAC resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable dye ligand resins include Blue Sepharose® resin (GE Healthcare, Piscataway, NJ) and Affi-gel® Blue resin (Bio-Rad Laboratories, Hercules, CA). Examples of suitable affinity resins include Protein A Sepharose® resin (*e.g.*, MabSelect, GE Healthcare, Piscataway, NJ) and lectin affinity resins, *e.g.*, Lentil Lectin Sepharose® resin (GE Healthcare, Piscataway, NJ), where the antibody bears appropriate lectin binding sites. Examples of suitable reversed phase resins include C4, C8, and C18 resins (Grace Vydac, Hesperia, CA).

[00181] Any suitable non-adsorptive chromatography resin may be utilized for purification. Examples of suitable non-adsorptive chromatography resins include, but are not limited to, SEPHADEX™ G-25, G-50, G-100, SEPHACRYL™ resins (*e.g.*, S-200 and S-300), SUPERDEX™ resins (*e.g.*, SUPERDEX™ 75 and SUPERDEX™ 200), BIO-GEL® resins (*e.g.*, P-6, P-10, P-30, P-60, and P-100), and others known to those of ordinary skill in the art.

Two-Step Process and One-Pot Process

[00182] In one embodiment, the conjugates of the present invention can be prepared as described in the U.S. Patent 7,811,572 and U.S. Patent Application Publication No. 2006/0182750. The process comprises the steps of (a) contacting the antibody of the present invention with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1) to covalently attach the linker (*i.e.*, Ab-SMCC, Ab-SPDB or Ab-CX1-1) to the antibody and thereby prepare a first mixture comprising the antibody having the linker bound thereto; (b) optionally subjecting the first mixture to a purification process to prepare a purified first mixture of the antibody having the linker bound thereto; (c) conjugating the drug (*e.g.*, DM1 or DM4) to the antibody having the linker bound thereto in the first mixture by reacting the antibody having the linker bound thereto with the drug (*e.g.*, DM1 or DM4) in a solution having a pH of about 4 to about 9 to prepare a second mixture comprising (i) conjugate (*e.g.*, Ab-MCC-DM1, Ab-SPDB-DM4 or Ab-CX1-1-DM1), (ii) free drug (*e.g.*, DM1 or DM4); and (iii) reaction by-products; and (d) subjecting the second mixture to a purification process to purify the conjugate from the other components of the second mixture. Alternatively, the purification step (b) can be omitted. Any purification methods described herein can be used for steps (b) and (d). In one embodiment, TFF is used for both steps (b) and (d). In another embodiment, TFF is used for step (b) and absorptive chromatography (*e.g.*, CHT) is used for step (d).

One-Step Reagent and In-situ Process

[00183] In one embodiment, the conjugates of the present invention can be prepared by conjugating pre-formed drug-linker compound (*e.g.*, SMCC-DM1, Sulfo-SMCC-DM1, SPDB-DM4 or CX1-1-DM1) to the antibody of the present invention, as described in U.S. Patent 6,441,163 and U.S. Patent Application Publication Nos. 2011/0003969 and 2008/0145374, followed by a purification step. Any purification methods described herein can be used. The drug-linker compound is prepared by reacting the drug (*e.g.*, DM1 or DM4) with the cross-linking agent (*e.g.*, SMCC, Sulfo-SMCC, SPDB, Sulfo-SPDB or CX1-1). The drug-linker compound (*e.g.*, SMCC-DM1, Sulfo-SMCC-DM1, SPDB-DM4 or CX1-1-DM1) is optionally subjected to purification before being conjugated to the antibody.

4. Characterization and Selection of Desirable Antibodies and Antibody Drug Conjugates

[00184] The antibodies, antibody fragments (*e.g.*, antigen binding fragments) or antibody drug conjugates of the present invention can be characterized and selected for their physical/chemical properties and/or biological activities by various assays known in the art.

[00185] For example, an antibody of the invention can be tested for its antigen binding activity by known methods such as ELISA, FACS, Biacore® or Western blot.

[00186] Transgenic animals and cell lines are particularly useful in screening antibody drug conjugates (ADCs) that have potential as prophylactic or therapeutic treatments of cancer overexpression of tumor-associated antigens and cell surface receptors. Screening for a useful ADC may involve administering a candidate ADC over a range of doses to the transgenic animal, and assaying at various time points for the effect(s) of the ADC on the disease or disorder being evaluated. Alternatively, or additionally, the drug can be administered prior to or simultaneously with exposure to an inducer of the disease, if applicable. The candidate ADC may be screened serially and individually, or in parallel under medium or high-throughput screening format.

[00187] One embodiment is a screening method comprising (a) transplanting cells from a stable cancer cell line or human patient tumor expressing FGFR2 or FGFR4 (*e.g.*, a breast cancer cell line or tumor fragment, a gastric cancer cell line or tumor fragment) into a non-human animal, (b) administering an ADC drug candidate to the non-human animal and (c) determining the ability of the candidate to inhibit the growth of tumors from the transplanted cell line. The invention also encompasses a method of screening ADC candidates for the treatment of a disease or disorder characterized by the overexpression of FGFR2 or FGFR4 comprising (a) contacting cells from a stable cancer cell line expressing FGFR2 or FGFR4 with a drug candidate, and (b) evaluating the ability of the ADC candidate to inhibit the growth of the stable cell line.

[00188] One embodiment is a screening method comprising (a) contacting cells from a stable cancer cell line expressing FGFR2 or FGFR4 with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to block ligand activation of FGFR2 or FGFR4. In another embodiment the ability of the ADC candidate to block ligand-stimulated tyrosine phosphorylation is evaluated.

[00189] Another embodiment is a screening method comprising (a) contacting cells from a stable cancer cell line expressing FGFR2 or FGFR4 with an ADC drug candidate and (b) evaluating the ability of the ADC candidate to induce cell death. In one embodiment the ability of the ADC candidate to induce apoptosis is evaluated.

[00190] In one embodiment, candidate ADC are screened by being administered to the transgenic animal over a range of doses, and evaluating the animal's physiological response to the compounds over time. In some cases, it may be appropriate to administer the compound in conjunction with co-factors that would enhance the efficacy of the compound. If cell lines derived from the subject transgenic animals are used to screen for ADCs useful in treating various disorders associated with overexpression of FGFR2 or FGFR4, the test ADCs are added to the cell culture medium at an appropriate time, and the cellular response to the ADCs is evaluated over time using the appropriate biochemical and/or histological assays.

[00191] Thus, the present invention provides assays for identifying ADC which specifically target and bind to both FGFR2 and FGFR4, the overexpression and amplification of which on the tumor cells is correlated with abnormal cellular function. In one embodiment, the present invention provides ADCs comprising an anti-FGFR2/4 antibody or antibody fragment (e.g., antigen binding fragment) that have the following attributes: affinity to human FGFR2 of less than 10 nM, following conjugation to MCC-DM1 or SPDB-DM4, ability to inhibit growth of FGFR2/4 amplified and/or overexpressing cells with an IC₅₀ of less than 2 nM, a slow clearance rate, for example, less than 45 ml/d/kg in a mouse following a single 3 mg/kg IV dose.

FGFR2/4 Antibodies

[00192] The present invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to human FGFR2 and FGFR4 (FGFR2/4). Antibodies or antibody fragments (*e.g.*, antigen binding fragments) of the invention include, but are not limited to, the human monoclonal antibodies or fragments thereof, isolated as described, in the Examples (see Section 6 below).

[00193] The present invention in certain embodiments provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind FGFR2/4, said antibodies or antibody fragments (*e.g.*, antigen binding fragments) comprise a VH domain having an amino acid sequence of SEQ ID NO: 7, 27, 47, 67, 87, or 107. The present invention in certain embodiments also provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to FGFR2/4, said antibodies or antibody fragments (*e.g.*, antigen binding fragments) comprise a VH CDR having an amino acid sequence of any one of the VH CDRs listed in Table 1, *infra*. In particular embodiments, the invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to FGFR2/4, said antibodies comprising (or alternatively, consist of) one, two, three, four, five or more VH CDRs having an amino acid sequence of any of the VH CDRs listed in Table 1, *infra*.

[00194] The present invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to FGFR2/4, said antibodies or antibody fragments (*e.g.*, antigen binding fragments) comprise a VL domain having an amino acid sequence of SEQ ID NO: 17, 37, 57, 77, 97, or 117. The present invention also provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to FGFR2/4, said antibodies or antibody fragments (*e.g.*, antigen binding fragments) comprise a VL CDR having an amino acid sequence of any one of the VL CDRs listed in Table 1, *infra*. In particular, the invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to FGFR2/4, said antibodies or antibody fragments (*e.g.*, antigen binding fragments) comprise (or alternatively, consist of) one, two, three or more VL CDRs having an amino acid sequence of any of the VL CDRs listed in Table 1, *infra*.

[00195] Other antibodies or antibody fragments (*e.g.*, antigen binding fragments) of the invention include amino acids that have been mutated, yet have at least 60, 70, 80, 90 or 95 percent identity in the CDR regions with the CDR regions depicted in the sequences described in

Table 1. In some embodiments, it includes mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the CDR regions when compared with the CDR regions depicted in the sequence described in Table 1.

[00196] The present invention also provides nucleic acid sequences that encode VH, VL, the full length heavy chain, and the full length light chain of the antibodies that specifically bind to FGFR2/4. Such nucleic acid sequences can be optimized for expression in mammalian cells.

Table 1. Examples of anti-FGFR2/4 Antibodies of the Present Invention

mAb 12425		
SEQ ID NO: 1	HCDR1 (Kabat)	DYAMS
SEQ ID NO: 2	HCDR2 (Kabat)	VIEGDGSYTHYADSVKVG
SEQ ID NO: 3	HCDR3 (Kabat)	EKTYSSAFDY
SEQ ID NO: 4	HCDR1 (Chothia)	GFTFSDY
SEQ ID NO: 5	HCDR2 (Chothia)	EGDGSY
SEQ ID NO: 6	HCDR3 (Chothia)	EKTYSSAFDY
SEQ ID NO: 7	VH	QVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLE WVSVIEGDGSYTHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCAREKTYSSAFDYWGQGTLVTVSS
SEQ ID NO: 8	DNA VH	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAGCCTGGC GGTAGCCTGAGACTGAGCTGTGCCGCTCCGGCTTCACCTTAGCG ACTACGCTATGAGCTGGGTCCGACAGGCCCTGGCAAGGGACTGG AATGGGTGTCAGTGATCGAGGGCGACGGTAGCTACACTCACTACG CCGATAGCGTGAAGGGCCGGTTCACTATCTCTAGGGACA ACTCTAA GAACACCCTGTACCTGCAGATGAACTCACTGAGAGCCGAGGACACC GCCGTCTACTACTGCGCTAGAGAAAAGACCTACTCTAGCGCCTTCG ACTACTGGGGCCAGGGCACCCTGGTCACCGTGT CATCA
SEQ ID NO: 9	Heavy Chain	QVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLE WVSVIEGDGSYTHYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCAREKTYSSAFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTGPS SSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
SEQ ID NO: 10	DNA Heavy Chain	CAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAGCCTGGC GGTAGCCTGAGACTGAGCTGTGCCGCTCCGGCTTCACCTTAGCG ACTACGCTATGAGCTGGGTCCGACAGGCCCTGGCAAGGGACTGG

		AATGGGTGTCAGTGATCGAGGGCGACGGTAGCTACACTCACTACG CCGATAGCGTGAAGGGCCGGTTCCTACTATCTCTAGGGACAACCTCTAA GAACACCCTGTACCTGCAGATGAACTCACTGAGAGCCGAGGACACC GCCGTCTACTACTGCGCTAGAGAAAAGACCTACTCTAGCGCCTTCG ACTACTGGGGCCAGGGCACCTGGTCACCGTGTCATCAGCTAGCAC TAAGGGCCCAAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTACT TCCGGCGGAACTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTCC CCGAGCCCGTGACAGTGTCTGGAACCTCTGGGGCTCTGACTTCCGG CGTGCACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGC CTGAGCAGCGTGGTGACAGTGCCCTCCAGCTCTCTGGGAACCCAGA CCTATATCTGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGA CAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCC CCCCTGCCAGCTCCAGAACTGCTGGGAGGGCCTTCCGTGTTCTG TTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCAGGACCCCCG AGGTGACCTGCGTGGTGGTGGACGTGTCCACGAGGACCCAGAGG TGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCA AGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGG TGTCCGTGCTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAG AATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCAGCCCCAATCGA AAAGACAATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGT GTACACCCTGCCCCCAGCCGGGAGGAGATGACCAAGAACCAGGT GTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCAGCGATATCGCC GTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACCTACAAGACC ACCCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCTGTACAGCA AGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTCA GCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAGAA GTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 11 (Kabat)	LCDR1	RASQDISSDLN
SEQ ID NO: 12 (Kabat)	LCDR2	DASNLQS
SEQ ID NO: 13 (Kabat)	LCDR3	QQHYSPSHT
SEQ ID NO: 14 (Chothia)	LCDR1	SQDISSD
SEQ ID NO: 15 (Chothia)	LCDR2	DAS
SEQ ID NO: 16 (Chothia)	LCDR3	HYSPSH
SEQ ID NO: 17	VL	DIQMTQSPSSLSASVGDRTITCRASQDISSDLNWWYQQKPGKAPKLLIY DASNLQSGVPSRFSGSGSGTDFTLTISLQPEDFAVYYCQQHYSPSHTF GQGTKVEIK
SEQ ID NO: 18	DNA VL	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCCTCAGTGG GCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGGACATCTCTAG CGACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCTAAGCT GCTGATCTACGACGCCTCTAACCTGCAGAGCGGCGTGCCCTCTAGG

		TTTAGCGGTAGCGGCTCAGGCACCGACTTTACCCTGACTATCTCTAG CCTGCAGCCCGAGGACTTCGCCGTCTACTACTGTCAGCAGCACTAT AGCCCTAGTCACACCTTCGGCCAGGGCACTAAGGTCGAGATTAAG
SEQ ID NO: 19	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQDISDLNWWYQQKPGKAPKLLIY DASNLQSGVPSRFSGSGSGTDFTLTISLQPEDFAVYYCQQHYSPSHTF GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
SEQ ID NO 20:	DNA Light Chain	GATATTGAGATGACTCAGTCACCTAGTAGCCTGAGCGCCTCAGTGG GCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGGACATCTCTAG CGACCTGAACTGGTATCAGCAGAAGCCCGGCAAGGCCCTAAGCT GCTGATCTACGACGCCTCTAACCTGCAGAGCGGCGTGCCCTCTAGG TTTAGCGGTAGCGGCTCAGGCACCGACTTTACCCTGACTATCTCTAG CCTGCAGCCCGAGGACTTCGCCGTCTACTACTGTCAGCAGCACTAT AGCCCTAGTCACACCTTCGGCCAGGGCACTAAGGTCGAGATTAAGC GTACGGTGGCCGCTCCCAGCGTGTTTCATCTTCCCCCCCAGCGACGA GCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAA CTTCTACCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGC CCTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGAGCAGGACA GCAAGGACTCCACCTACAGCCTGAGCAGCACCTGACCCTGAGCAA GGCCGACTACGAGAAGCATAAGGTGTACGCCTGCGAGGTGACCCA CCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACAGGGGCGA GTGC
mAb 14629		
SEQ ID NO: 21	HCDR1 (Kabat)	SYAIS
SEQ ID NO: 22	HCDR2 (Kabat)	YISPYMGETHYAQRFQG
SEQ ID NO: 23	HCDR3 (Kabat)	ESYEYFDI
SEQ ID NO: 24	HCDR1 (Chothia)	GGTFSSY
SEQ ID NO: 25	HCDR2 (Chothia)	SPYMGE
SEQ ID NO: 26	HCDR3 (Chothia)	ESYEYFDI
SEQ ID NO: 27	VH	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAIWVRQAPGQGLE WMGYISPYMGETHYAQRFQGRVTITADESTSTAYMELSSLRSEDTAV YYCARESYEYFDIWGQGTLVTVSS
SEQ ID NO: 28	DNA VH	CAGGTGCAGCTGGTGCAGTCAGGCGCCGAAGTGAAGAAACCCGGC TCTAGCGTGAAGGTGTCCTGTAAAGCCTCCGGCGGCACCTTCTCTA GCTACGCTATTAGCTGGGTCCGACAGGCCCCAGGACAGGGCCTGG AATGGATGGGCTATATTAGCCCCTATATGGGCGAGACTCACTACGC TCAGCGGTTTTCAGGGTAGAGTGACTATCACCGCCGACGAGTCTACT AGCACCGCCTATATGGAAGTGTCTAGCCTGAGATCAGAGGACACCG CCGTCTACTACTGCGCTAGAGAGTCCTACGAGTACTTCGATATCTGG

		GGCCAGGGCACCTGGTCACCGTGTCATCA
SEQ ID NO: 29	Heavy Chain	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLE WMGYISPYMGETHYAQRFRVTITADESTSTAYMELSSLRSEDVAV YYCARESYEYFDIWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFCFSVMHEA LHNHYTQKSLSLSPGK
SEQ ID NO: 30	DNA Heavy Chain	CAGGTGCAGCTGGTGCAGTCAGGCGCCGAAGTGAAGAAACCCGGC TCTAGCGTGAAGGTGTCCTGTAAAGCCTCCGGCGGCACCTTCTCTA GCTACGCTATTAGCTGGGTCCGACAGGCCCCAGGACAGGGCCTGG AATGGATGGGCTATATTAGCCCCTATATGGGCGAGACTCACTACGC TCAGCGGTTTCAGGGTAGAGTGACTATCACCGCCGACGAGTCTACT AGCACCGCCTATATGGAAGTGTCTAGCCTGAGATCAGAGGACACCG CCGTCTACTACTGCGCTAGAGAGTCCTACGAGTACTTCGATATCTGG GGCCAGGGCACCTGGTCACCGTGTCATCAGCTAGCACTAAGGGCC CAAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTACTTCCGGCGG AACTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTCCCCGAGCCC GTGACAGTGTCTGGAAGTCTGGGGCTCTGACTTCCGGCGTGCACA CCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAGCCTGAGCAG CGTGGTGACAGTGCCCTCCAGCTCTCTGGGAACCCAGACCTATATC TGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGGACAAGAGA GTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCCCCCCTGCC CAGCTCCAGAACTGCTGGGAGGGCCTTCCGTGTTCTGTCCCCCCC AAGCCCAAGGACACCCTGATGATCAGCAGGACCCCCGAGGTGACC TGCCTGGTGGTGGACGTGTCCACGAGGACCCAGAGGTGAAGTTC AACTGGTACGTGGACGGCGTGGAGGTGCACAACGCCAAGACCAAG CCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTGGTGTCCGTG CTGACCGTGCTGCACCAGGACTGGCTGAACGGCAAAGAATACAAG TGCAAAGTCTCCAACAAGGCCCTGCCAGCCCCAATCGAAAAGACAA TCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAGGTGTACACCC TGCCCCCAGCCGGGAGGAGATGACCAAGAACCAGGTGTCCCTGA CCTGTCTGGTGAAGGGCTTCTACCCCAGCGATATCGCCGTGGAGTG GGAGAGCAACGGCCAGCCCGAGAACAACCTACAAGACCACCCCCC AGTGCTGGACAGCGACGGCAGCTTCTCCTGTACAGCAAGCTGACC GTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTTTCAGCTGCAGC GTGATGCACGAGGCCCTGCACAACCACTACACCCAGAAGTCCCTGA GCCTGAGCCCCGGCAAG

SEQ ID NO: 31 (Kabat)	LCDR1	RASQSI SNDLA
SEQ ID NO: 32 (Kabat)	LCDR2	ATSILQS
SEQ ID NO: 33 (Kabat)	LCDR3	LQYYDYSYT
SEQ ID NO: 34 (Chothia)	LCDR1	SQSI SND
SEQ ID NO: 35 (Chothia)	LCDR2	ATS
SEQ ID NO: 36 (Chothia)	LCDR3	YYDYSY
SEQ ID NO: 37	VL	DIQMTQSPSSLSASVGDRVITTCRASQSI SNDLAWYQQKPGKAPKLLIY ATSILQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQYYDYSYTFG QGTKVEIK
SEQ ID NO: 38	DNA VL	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCCTCAGTGG GCGATAGAGTGACTATCACCTGTAGAGCCTCCCAGTCTATCTCTAAC GACCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCTAAGCTG CTGATCTACGCTACCTCTATCCTGCAGAGCGGCGTGCCCTCTAGGTT TAGCGGTAGCGGCTCAGGCACCGACTTTACCCTGACTATCTCTAGC CTGCAGCCCGAGGACTTCGCTACCTACTACTGCCTGCAGTACTACG ACTACTCCTACACCTTCGGCCAGGGCACTAAGGTCGAGATTAAG
SEQ ID NO: 39	Light Chain	DIQMTQSPSSLSASVGDRVITTCRASQSI SNDLAWYQQKPGKAPKLLIY ATSILQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQYYDYSYTFG QGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
SEQ ID NO: 40	DNA Light Chain	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCCTCAGTGG GCGATAGAGTGACTATCACCTGTAGAGCCTCCCAGTCTATCTCTAAC GACCTGGCCTGGTATCAGCAGAAGCCCGGCAAGGCCCTAAGCTG CTGATCTACGCTACCTCTATCCTGCAGAGCGGCGTGCCCTCTAGGTT TAGCGGTAGCGGCTCAGGCACCGACTTTACCCTGACTATCTCTAGC CTGCAGCCCGAGGACTTCGCTACCTACTACTGCCTGCAGTACTACG ACTACTCCTACACCTTCGGCCAGGGCACTAAGGTCGAGATTAAGCG TACGGTGGCCGCTCCCAGCGTGTTTCATCTTCCCCCCCAGCGACGAG CAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAAC TTCTACCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCC CTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGAGCAGGACAG CAAGGACTCCACCTACAGCCTGAGCAGCACCTGACCCTGAGCAAG GCCGACTACGAGAAGCATAAGGTGTACGCCTGCGAGGTGACCCAC CAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACAGGGGCGAG TGC
mAb 20562		
SEQ ID NO: 41	HCDR1 (Kabat)	DYAMS
SEQ ID NO: 42	HCDR2 (Kabat)	VIEGDASYTHYADSVRG
SEQ ID NO: 43	HCDR3 (Kabat)	ERTYSSAFDY

SEQ ID NO: 44	HCDR1 (Chothia)	GFTFSDY
SEQ ID NO: 45	HCDR2 (Chothia)	EGDASY
SEQ ID NO: 46	HCDR3 (Chothia)	ERTYSSAFDY
SEQ ID NO: 47	VH	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLE WVSVIEGDASYTHYADSVRGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARERTYSSAFDYWGQGTLVTVSS
SEQ ID NO: 48	DNA VH	GAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAGCCTGGC GGTAGCCTGAGACTGAGCTGCGCTGCTAGTGGCTTCACCTTTAGCG ACTACGCTATGAGCTGGGTCAGACAGGCCCTGGTAAAGGCCTGG AGTGGGTCAGCGTGATCGAGGGCGACGCTAGTTAACTCACTACG CCGATAGCGTCAGAGGCCGGTTCCTATCTCTAGGGATAACTCTAA GAACACCCTGTACCTGCAGATGAATAGCCTGAGAGCCGAGGACAC CGCCGTCTACTACTGCGCTAGAGAGCGGACCTACTCTAGCGCCTTC GACTACTGGGGTCAGGGCACCCCTGGTCACCGTGTCTAGC
SEQ ID NO: 49	Heavy Chain	EVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLE WVSVIEGDASYTHYADSVRGRFTISRDN SKNTLYLQMNSLRAEDTAVY YCARERTYSSAFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAA LGCLVKDYFPEPVTWSNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPS VFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHN AKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
SEQ ID NO: 50	DNA Heavy Chain	GAGGTGCAGCTGCTGGAATCAGGCGGCGGACTGGTGCAGCCTGGC GGTAGCCTGAGACTGAGCTGCGCTGCTAGTGGCTTCACCTTTAGCG ACTACGCTATGAGCTGGGTCAGACAGGCCCTGGTAAAGGCCTGG AGTGGGTCAGCGTGATCGAGGGCGACGCTAGTTAACTCACTACG CCGATAGCGTCAGAGGCCGGTTCCTATCTCTAGGGATAACTCTAA GAACACCCTGTACCTGCAGATGAATAGCCTGAGAGCCGAGGACAC CGCCGTCTACTACTGCGCTAGAGAGCGGACCTACTCTAGCGCCTTC GACTACTGGGGTCAGGGCACCCCTGGTCACCGTGTCTAGCGCTAGCA CTAAGGGCCCAAGTGTGTTTCCCCTGGCCCCCAGCAGCAAGTCTAC TTCCGGCGGAACTGCTGCCCTGGGTTGCCTGGTGAAGGACTACTTC CCCGAGCCCGTGACAGTGTCTGGAAGTCTGGGGCTCTGACTTCCG GCGTGACACCTTCCCCGCCGTGCTGCAGAGCAGCGGCCTGTACAG CCTGAGCAGCGTGGTGACAGTGCCCTCCAGCTCTCTGGGAACCCAG ACCTATATCTGCAACGTGAACCACAAGCCCAGCAACACCAAGGTGG ACAAGAGAGTGGAGCCCAAGAGCTGCGACAAGACCCACACCTGCC CCCCCTGCCAGCTCCAGAACTGCTGGGAGGGCCTTCCGTGTTCTT GTTCCCCCCCCAAGCCCAAGGACACCCTGATGATCAGCAGGACCCCC

		GAGGTGACCTGCGTGGTGGTGGACGTGTCCCACGAGGACCCAGAG GTGAAGTTCAACTGGTACGTGGACGGCGTGGAGGTGCACAACGCC AAGACCAAGCCCAGAGAGGAGCAGTACAACAGCACCTACAGGGTG GTGTCCGTGCTGACCGTGTGCACCAGGACTGGCTGAACGGCAAA GAATACAAGTGCAAAGTCTCCAACAAGGCCCTGCCAGCCCCAATCG AAAAGACAATCAGCAAGGCCAAGGGCCAGCCACGGGAGCCCCAG GTGTACACCCTGCCCCCAGCCGGGAGGAGATGACCAAGAACCAG GTGTCCCTGACCTGTCTGGTGAAGGGCTTCTACCCAGCGATATCG CCGTGGAGTGGGAGAGCAACGGCCAGCCCGAGAACAACACTACAAGA CCACCCCCCAGTGCTGGACAGCGACGGCAGCTTCTTCCTGTACAG CAAGCTGACCGTGGACAAGTCCAGGTGGCAGCAGGGCAACGTGTT CAGCTGCAGCGTGATGCACGAGGCCCTGCACAACCACTACACCCAG AAGTCCCTGAGCCTGAGCCCCGGCAAG
SEQ ID NO: 51	LCDR1 (Kabat)	RASQDISSDLN
SEQ ID NO: 52	LCDR2 (Kabat)	DASNLQS
SEQ ID NO: 53	LCDR3 (Kabat)	QQHYSPSHT
SEQ ID NO: 54	LCDR1 (Chothia)	SQDISSD
SEQ ID NO: 55	LCDR2 (Chothia)	DAS
SEQ ID NO: 56	LCDR3 (Chothia)	HYSPSH
SEQ ID NO: 57	VL	DIQMTQSPSSLSASVGDRVITICRASQDISSDLNWWYQQKPGKAPKLLIY DASNLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHYSPSHTF GQGTKVEIK
SEQ ID NO: 58	DNA VL	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAGTGTGG GCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGGATATCTCTAG CGACCTGAACTGGTATCAGCAGAAGCCCGGTAAAGCCCCTAAGCTG CTGATCTACGACGCCTCTAACCTGCAGTCAGGCGTGCCCTCTAGGTT TAGCGGTAGCGGTAGTGGCACCGACTTCACCCTGACTATCTCTAGC CTGCAGCCCGAGGACTTCGCTACCTACTACTGTCAGCAGCACTATA GCCCTAGTCACACCTTCGGTCAGGGCACTAAGGTCGAGATTAAG
SEQ ID NO: 59	Light Chain	DIQMTQSPSSLSASVGDRVITICRASQDISSDLNWWYQQKPGKAPKLLIY DASNLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHYSPSHTF GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACE VTHQGLSPVTKSFNRGEC
SEQ ID NO: 60	DNA Light Chain	GATATTCAGATGACTCAGTCACCTAGTAGCCTGAGCGCTAGTGTGG GCGATAGAGTGACTATCACCTGTAGAGCCTCTCAGGATATCTCTAG CGACCTGAACTGGTATCAGCAGAAGCCCGGTAAAGCCCCTAAGCTG CTGATCTACGACGCCTCTAACCTGCAGTCAGGCGTGCCCTCTAGGTT TAGCGGTAGCGGTAGTGGCACCGACTTCACCCTGACTATCTCTAGC CTGCAGCCCGAGGACTTCGCTACCTACTACTGTCAGCAGCACTATA

		GCCCTAGTCACACCTTCGGTCAGGGCACTAAGGTCGAGATTAAGCG TACGGTGGCCGCTCCCAGCGTGTTTCATCTTCCCCCCCAGCGACGAG CAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAAC TTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGCC CTGCAGAGCGGCAACAGCCAGGAGAGCGTCACCGAGCAGGACAG CAAGGACTCCACCTACAGCCTGAGCAGCACCTGACCCTGAGCAAG GCCGACTACGAGAAGCATAAGGTGTACGCCTGCGAGGTGACCCAC CAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACAGGGGCGAG TGC
mAb 20811		
SEQ ID NO: 61	HCDR1 (Kabat)	DYAMS
SEQ ID NO: 62	HCDR2 (Kabat)	TIEGDSNYIEYADSVKG
SEQ ID NO: 63	HCDR3 (Kabat)	ERTYSSAFDY
SEQ ID NO: 64	HCDR1 (Chothia)	GFTFSDY
SEQ ID NO: 65	HCDR2 (Chothia)	EGDSNY
SEQ ID NO: 66	HCDR3 (Chothia)	ERTYSSAFDY
SEQ ID NO: 67	VH	QVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLE WVSTIEGDSNYIEYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYY CARERTYSSAFDYWGQGTLVTVSS
SEQ ID NO: 68	DNA VH	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCCGGGT GGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTACCTTTTCTG ACTACGCTATGTCTTGGGTGCGCCAGGCCCGGGCAAAGGTCTCGA GTGGGTTTCCACTATCGAAGGTGACAGCAACTACATCGAATATGCG GATAGCGTGAAAGGCCGCTTTACCATCAGCCGCGATAATTCGAAAA ACACCCTGTATCTGCAAATGAACAGCCTGCGTGCGGAAGATACGGC CGTGTATTATTGCGCGCGTGAACGTACTTACTCTTCTGCTTTCGATT ACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
SEQ ID NO: 69	Heavy Chain	QVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAPGKGLE WVSTIEGDSNYIEYADSVKGRFTISRDN SKNTLYLQMNSLRAEDTAVYY CARERTYSSAFDYWGQGTLVTVSSASTKGPSVFPLAPSSKSTSGGTAAL GCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPS SLGTQTYICNVNHKPSNTKVDKRVEPKSCDKHTHTCPPCPAPELLGGPSV FLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESN GQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEA LHNHYTQKSLSLSPGK
SEQ ID NO: 70	DNA Heavy Chain	CAGGTGCAATTGCTGGAAAGCGGCGGTGGCCTGGTGCAGCCGGGT GGCAGCCTGCGTCTGAGCTGCGCGGCGTCCGGATTACCTTTTCTG ACTACGCTATGTCTTGGGTGCGCCAGGCCCGGGCAAAGGTCTCGA

		GTGGGTTTCCACTATCGAAGGTGACAGCAACTACATCGAATATGCG GATAGCGTGAAAGGCCGCTTTACCATCAGCCGCGATAATTCGAAAA ACACCCTGTATCTGCAAATGAACAGCCTGCGTGCGGAAGATACGGC CGTGTATTATTGCGCGCGTGAACGTACTIONACTCTTCTGCTTTCGATT ACTGGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAA GGGTCCATCGGTCTTCCCCCTGGCACCCTCCTCCAAGAGCACCTCTG GGGGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCG AACCGGTGACGGTGTCGTGGAACCTCAGGCGCCCTGACCAGCGGCG TGCACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTC AGCAGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCT ACATCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAA GAGAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCACCG TGCCACAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCC CCAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTC ACATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAG TTCAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACA AAGCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAG CGTCCTCACCGTCTGCACCAGGACTGGCTGAATGGCAAGGAGTAC AAGTGCAAGGTCTCCAACAAAGCCCTCCCAGCCCCCATCGAGAAAA CCATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACAC CCTGCCCCCATCCCGGGAGGAGATGACCAAGAACCAGGTCAGCCT GACCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAG TGGGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCT CCCGTGCTGGACTCCGACGGCTCCTTCTTCTCTACAGCAAGCTCAC CGTGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTC CGTGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTC TCCCTGTCTCCGGGTAAA
SEQ ID NO: 71 (Kabat)	LCDR1	RASQDISSDLN
SEQ ID NO: 72 (Kabat)	LCDR2	DASNLQS
SEQ ID NO: 73 (Kabat)	LCDR3	HQWYSTLYT
SEQ ID NO: 74 (Chothia)	LCDR1	SQDISSD
SEQ ID NO: 75 (Chothia)	LCDR2	DAS
SEQ ID NO: 76 (Chothia)	LCDR3	WYSTLY
SEQ ID NO: 77	VL	DIQMTQSPSSLSASVGDRVITICRASQDISSDLNWWYQQKPGKAPKLLIY DASNLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCHQWYSTLYTF GQGTEKVEIK
SEQ ID NO: 78	DNA VL	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAGCGTG GGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGGACATTTCTT CTGACCTGAACTGGTACCAGCAGAAACCGGGCAAAGCGCCGAAAC TATTAATCTACGACGCTTCTAACCTGCAAAGCGGCGTGCCGAGCCG

		CTTTAGCGGCAGCGGATCCGGCACCGATTCACCCTGACCATTAGC TCTCTGCAACCGGAAGACTTTGCGACCTATTATTGCCATCAGTGGTA CTCTACTCTGTACACCTTTGGCCAGGGCACGAAAGTTGAAATTA
SEQ ID NO: 79	Light Chain	DIQMTQSPSSLSASVGDRVTITCRASQDISDLNWFYQQKPGKAPKLLIY DASNLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCHQWYSTLYTF GQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQ WKVDNALQSGNSQESVTEQDSKDSYLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC
SEQ ID NO: 80	DNA Light Chain	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAGCGTG GGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGGACATTTCTT CTGACCTGAACTGGTACCAGCAGAAACCGGGCAAAGCGCCGAAAC TATTAATCTACGACGCTTCTAACCTGCAAAGCGGCGTGCCGAGCCG CTTTAGCGGCAGCGGATCCGGCACCGATTCACCCTGACCATTAGC TCTCTGCAACCGGAAGACTTTGCGACCTATTATTGCCATCAGTGGTA CTCTACTCTGTACACCTTTGGCCAGGGCACGAAAGTTGAAATTA CGTACGGTGGCCGCTCCCAGCGTGTTTCATCTTCCCCCCCAGCGACG AGCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACA ACTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACG CCCTGCAGAGCGGCAACAGCCAGGAAAGCGTCACCGAGCAGGACA GCAAGGACTCCACCTACAGCCTGAGCAGCACCTGACCCTGAGCAA GGCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCA CCAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGGCGA GTGT
mAb 12422		
SEQ ID NO: 81	HCDR1 (Kabat)	SYAIS
SEQ ID NO: 82	HCDR2 (Kabat)	YISPYMGETHYAQKFQG
SEQ ID NO: 83	HCDR3 (Kabat)	ESYEYFDI
SEQ ID NO: 84	HCDR1 (Chothia)	GGTFSSY
SEQ ID NO: 85	HCDR2 (Chothia)	SPYMGE
SEQ ID NO: 86	HCDR3 (Chothia)	ESYEYFDI
SEQ ID NO: 87	VH	QVQLVQSGAEVKKPGSSVKVCKASGGTFSSYAIWVRQAPGQGLE WMGYISPYMGETHYAQKFQGRVTITADESTSTAYMELSSLRSEDAVY YCARESYEYFDIWGQGTLLTVSS
SEQ ID NO: 88	DNA VH	CAGGTGCAATTGGTGCAGAGCGGTGCCGAAGTGAAAAAACCGGC AGCAGCGTGAAAGTTAGCTGCAAAGCATCCGGAGGGACGTTTAGC AGCTATGCGATTAGCTGGGTGCGCCAGGCCCGGGCCAGGGCCTC GAGTGGATGGGCTACATCTCTCCGTACATGGGCGAAACTCATTACG CCCAGAAATTTCAGGGGCCGGGTGACCATTACCGCCGATGAAAGCAC CAGCACCGCCTATATGGAAGTGAAGCAGCCTGCGCAGCGAAGATAC GGCCGTGTATTATTGCGCGCGTGAATCTTACGAATACTTCGACATCT

		GGGGCCAAGGCACCCTGGTGACTGTTAGCTCA
SEQ ID NO: 89	Heavy Chain	QVQLVQSGAEVKKPGSSVKVSKASGGTFSSYAISWVRQAPGQGLE WMGYISPYMGETHYAQKFQGRVTITADESTSTAYMELSSLRSEDNAVY YCARESYEYFDIWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALG CLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSS LGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCPPCPAPELLGGPSVF LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAK TKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEAL HNHYTQKSLSLSPGK
SEQ ID NO: 90	DNA Heavy Chain	CAGGTGCAATTGGTGCAAGCGGTGCCGAAGTGAAAAAACCGGGC AGCAGCGTGAAAGTTAGCTGCAAAGCATCCGGAGGGACGTTTAGC AGCTATGCGATTAGCTGGGTGCGCCAGGCCCGGGCCAGGGCCTC GAGTGGATGGGCTACATCTCTCCGTACATGGGCGAAACTCATTACG CCCAGAAATTTAGGGCCGGGTGACCATTACCGCCGATGAAAGCAC CAGCACCGCCTATATGGAAGTGAAGCAGCCTGCGCAGCGAAGATAC GGCCGTGTATTATTGCGCGCGTGAATCTTACGAATACTTCGACATCT GGGGCCAAGGCACCCTGGTGACTGTTAGCTCAGCCTCCACCAAGG GTCCATCGGTCTTCCCCCTGGCACCCCTCCTCCAAGAGCACCTCTGGG GGCACAGCGGCCCTGGGCTGCCTGGTCAAGGACTACTTCCCCGAAC CGGTGACGGTGTCGTGGAAGTCAAGGCGCCCTGACCAGCGGCGTGC ACACCTTCCCGGCTGTCCTACAGTCCTCAGGACTCTACTCCCTCAGC AGCGTGGTGACCGTGCCCTCCAGCAGCTTGGGCACCCAGACCTACA TCTGCAACGTGAATCACAAGCCCAGCAACACCAAGGTGGACAAGA GAGTTGAGCCCAAATCTTGTGACAAAACCTCACACATGCCCACCGTG CCCAGCACCTGAACTCCTGGGGGGACCGTCAGTCTTCTCTTCCCCC CAAAACCCAAGGACACCCTCATGATCTCCCGGACCCCTGAGGTCAC ATGCGTGGTGGTGGACGTGAGCCACGAAGACCCTGAGGTCAAGTT CAACTGGTACGTGGACGGCGTGGAGGTGCATAATGCCAAGACAAA GCCGCGGGAGGAGCAGTACAACAGCACGTACCGGGTGGTCAGCGT CCTCACCGTCCTGCACCAGGACTGGCTGAATGGCAAGGAGTACAA GTGCAAGGTCTCCAACAAAGCCCTCCAGCCCCCATCGAGAAAACC ATCTCCAAAGCCAAAGGGCAGCCCCGAGAACCACAGGTGTACACCC TGCCCCCATCCCGGAGGAGATGACCAAGAACCAGGTGAGCCTGA CCTGCCTGGTCAAAGGCTTCTATCCCAGCGACATCGCCGTGGAGTG GGAGAGCAATGGGCAGCCGGAGAACAACCTACAAGACCACGCCTCC CGTGCTGGACTCCGACGGCTCCTTCTCCTCTACAGCAAGCTCACCG TGGACAAGAGCAGGTGGCAGCAGGGGAACGTCTTCTCATGCTCCG TGATGCATGAGGCTCTGCACAACCACTACACGCAGAAGAGCCTCTC CCTGTCTCCGGGTAAA

SEQ ID NO: 91 (Kabat)	LCDR1	RASQSI SNDLA
SEQ ID NO: 92 (Kabat)	LCDR2	ATSILQS
SEQ ID NO: 93 (Kabat)	LCDR3	LQYYDYSYT
SEQ ID NO: 94 (Chothia)	LCDR1	SQSI SND
SEQ ID NO: 95 (Chothia)	LCDR2	ATS
SEQ ID NO: 96 (Chothia)	LCDR3	YYDYSY
SEQ ID NO: 97	VL	DIQMTQSPSSLSASVGDRVITTCRASQSI SNDLAWYQQKPGKAPKLLIY ATSILQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQYYDYSYTFG QGTKVEIK
SEQ ID NO: 98	DNA VL	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAGCGTG GGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGTCTATTTCTA ACGACCTGGCTTGGTACCAGCAGAAACCGGGCAAAGCGCCGAAAC TATTAATCTACGCTACTTCTATCCTGCAAAGCGGCGTGCCGAGCCGC TTTAGCGGCAGCGGATCCGGCACCGATTTCACCCTGACCATTAGCT CTCTGCAACCGGAAGACTTTGCGACCTATTATTGCCTGCAGTACTAC GACTACTCTTACACCTTTGGCCAGGGCACGAAAGTTGAAATTA
SEQ ID NO: 99	Light Chain	DIQMTQSPSSLSASVGDRVITTCRASQSI SNDLAWYQQKPGKAPKLLIY ATSILQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQYYDYSYTFG QGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQW KVDNALQSGNSQESVTEQDSKDYSLSTLTLSKADYEKHKVYACEVT HQGLSSPVTKSFNRGEC
SEQ ID NO: 100	DNA Light Chain	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAGCGTG GGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGTCTATTTCTA ACGACCTGGCTTGGTACCAGCAGAAACCGGGCAAAGCGCCGAAAC TATTAATCTACGCTACTTCTATCCTGCAAAGCGGCGTGCCGAGCCGC TTTAGCGGCAGCGGATCCGGCACCGATTTCACCCTGACCATTAGCT CTCTGCAACCGGAAGACTTTGCGACCTATTATTGCCTGCAGTACTAC GACTACTCTTACACCTTTGGCCAGGGCACGAAAGTTGAAATTA GTACGGTGGCCGCTCCCAGCGTGTTTCATCTTCCCCCAGCGACGA GCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAA CTTCTACCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGC CCTGCAGAGCGGCAACAGCCAGGAAAGCGTCACCGAGCAGGACAG CAAGGACTCCACCTACAGCCTGAGCAGCACCTGACCCTGAGCAAG GCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCAC CAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAG TGT
mAb 12439		
SEQ ID NO: 101	HCDR1 (Kabat)	SYDIS
SEQ ID NO: 102	HCDR2 (Kabat)	WINPYNGGTNYAQKFQG
SEQ ID NO: 103	HCDR3 (Kabat)	EGSGMIVYPGWSYAFDY

SEQ ID NO: 104	HCDR1 (Chothia)	GYTFTSY
SEQ ID NO: 105	HCDR2 (Chothia)	NPYNGG
SEQ ID NO: 106	HCDR3(Chothia)	EGSGMIVYPGWSYAFDY
SEQ ID NO: 107	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDISWVRQAPGQGLE WMGWINPYNGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSEDTA VYYCAREGSGMIVYPGWSYAFDYWGQGTLVTVSS
SEQ ID NO: 108	DNA VH	CAGGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAAAAACCGGG TGCCAGCGTGAAAGTTAGCTGCAAAGCGTCCGGATATACCTTCACT TCTTACGACATCTCTTGGGTGCGCCAGGCCCGGGCCAGGGCCTCG AGTGGATGGGCTGGATCAACCCGTACAACGGCGGTACGAACTACG CGCAGAAATTTAGGGCCGGGTGACCATGACCCGTGATACCAGCAT TAGCACCGCGTATATGGAAGTGAAGCGTCTGCGTAGCGAAGATAC GGCCGTGTATTATTGCGCGCGTGAAGGTTCTGGTATGATCGTTTAC CCGGGTTGGTCTTACGCTTTCGATTACTGGGGCCAAGGCACCCTGG TGAAGTGTAGCTCA
SEQ ID NO: 109	Heavy Chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYDISWVRQAPGQGLE WMGWINPYNGGTNYAQKFQGRVTMTRDTSISTAYMELSRLRSEDTA VYYCAREGSGMIVYPGWSYAFDYWGQGTLVTVSSASTKGPSVFPLAP SSKSTSGGTAALGCLVKDYFPEPVTWSNSGALTSGVHTFPAVLQSSG LYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCK VSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQ GNVFSCSVMHEALHNHYTQKSLSLSPGK
SEQ ID NO: 110	DNA Heavy Chain	CAGGTGCAATTGGTGCAGAGCGGTGCGGAAGTGAAAAACCGGG TGCCAGCGTGAAAGTTAGCTGCAAAGCGTCCGGATATACCTTCACT TCTTACGACATCTCTTGGGTGCGCCAGGCCCGGGCCAGGGCCTCG AGTGGATGGGCTGGATCAACCCGTACAACGGCGGTACGAACTACG CGCAGAAATTTAGGGCCGGGTGACCATGACCCGTGATACCAGCAT TAGCACCGCGTATATGGAAGTGAAGCGTCTGCGTAGCGAAGATAC GGCCGTGTATTATTGCGCGCGTGAAGGTTCTGGTATGATCGTTTAC CCGGGTTGGTCTTACGCTTTCGATTACTGGGGCCAAGGCACCCTGG TGAAGTGTAGCTCAGCCTCCACCAAGGGTCCATCGGTCTTCCCCCTG GCACCCTCCTCAAGAGCACCTCTGGGGGCACAGCGGCCCTGGGCT GCCTGGTCAAGGACTACTTCCCCGAACCGGTGACGGTGTCTGTGGAA CTCAGGCGCCCTGACCAGCGGCGTGCACACCTTCCCGGCTGTCTTA CAGTCCTCAGGACTCTACTCCCTCAGCAGCGTGGTGACCGTGCCCTC CAGCAGCTTGGGCACCCAGACCTACATCTGCAACGTGAATCACAAG CCCAGCAACACCAAGGTGGACAAGAGAGTTGAGCCCAAATCTTGT GACAAAACCTCACACATGCCACCGTGCCAGCACCTGAACTCCTGG

		GGGGACCGTCAGTCTTCCTCTTCCCCCAAACCCAAGGACACCCTC ATGATCTCCCGGACCCCTGAGGTCACATGCGTGGTGGTGGACGTGA GCCACGAAGACCCTGAGGTCAAGTTCAACTGGTACGTGGACGGCG TGGAGGTGCATAATGCCAAGACAAAGCCGCGGGAGGAGCAGTACA ACAGCACGTACCGGGTGGTCAGCGTCCTCACCGTCCTGCACCAGGA CTGGCTGAATGGCAAGGAGTACAAGTGCAAGGTCTCCAACAAAGC CCTCCCAGCCCCATCGAGAAAACCATCTCCAAAGCCAAAGGGCAG CCCCGAGAACCACAGGTGTACACCCTGCCCCCATCCCGGGAGGAG ATGACCAAGAACCAGGTCAGCCTGACCTGCCTGGTCAAAGGCTTCT ATCCCAGCGACATCGCCGTGGAGTGGGAGAGCAATGGGCAGCCGG AGAACAATAACAAGACCACGCCTCCCGTGCTGGACTCCGACGGCTC CTTCTTCCTCTACAGCAAGCTCACCGTGGACAAGAGCAGGTGGCAG CAGGGGAACGTCTTCTCATGCTCCGTGATGCATGAGGCTCTGCACA ACCACTACACGCAGAAGAGCCTCTCCCTGTCTCCGGGTAAA
SEQ ID NO: 111 (Kabat)	LCDR1	RASQDISNDLG
SEQ ID NO: 112 (Kabat)	LCDR2	AASSLQS
SEQ ID NO: 113 (Kabat)	LCDR3	QQHYHTPNT
SEQ ID NO: 114 (Chothia)	LCDR1	SQDISND
SEQ ID NO: 115 (Chothia)	LCDR2	AAS
SEQ ID NO: 116 (Chothia)	LCDR3	HYHTPN
SEQ ID NO: 117	VL	DIQMTQSPSSLSASVGDRVITICRASQDISNDLGWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHYHTPNT FGQGTKVEIK
SEQ ID NO: 118	DNA VL	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAGCGTG GGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGGACATTTCTA ACGACCTGGGTTGGTACCAGCAGAAACCGGGCAAAGCGCCGAAAC TATTAATCTACGCTGCTTCTTCTCTGCAAAGCGGCGTGCCGAGCCGC TTTAGCGGCAGCGGATCCGGCACCGATTTACCCTGACCATTAGCT CTCTGCAACCGGAAGACTTTGCGACCTATTATTGCCAGCAGCATTAC CATACTCCGAACACCTTTGGCCAGGGCACGAAAGTTGAAATTA
SEQ ID NO: 119	Light Chain	DIQMTQSPSSLSASVGDRVITICRASQDISNDLGWYQQKPGKAPKLLI YAASSLQSGVPSRFSGSGSGTDFTLTISLQPEDFATYYCQQHYHTPNT FGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKV QWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYAC EVTHQGLSSPVTKSFNRGEC

SEQ ID NO: 120	DNA Light Chain	GATATCCAGATGACCCAGAGCCCGAGCAGCCTGAGCGCCAGCGTG GGCGATCGCGTGACCATTACCTGCAGAGCCAGCCAGGACATTTCTA ACGACCTGGGTTGGTACCAGCAGAAACCGGGCAAAGCGCCGAAAC TATTAATCTACGCTGCTTCTTCTCTGCAAAGCGGCGTGCCGAGCCGC TTTAGCGGCAGCGGATCCGGCACCGATTTCACCCTGACCATTAGCT CTCTGCAACCGGAAGACTTTGCGACCTATTATTGCCAGCAGCATTAC CATACTCCGAACACCTTTGGCCAGGGGCACGAAAGTTGAAATTAAAC GTACGGTGGCCGCTCCCAGCGTGTTTCATCTTCCCCCCCAGCGACGA GCAGCTGAAGAGCGGCACCGCCAGCGTGGTGTGCCTGCTGAACAA CTTCTACCCCCGGGAGGCCAAGGTGCAGTGGAAGGTGGACAACGC CCTGCAGAGCGGCAACAGCCAGGAAAGCGTCACCGAGCAGGACAG CAAGGACTCCACCTACAGCCTGAGCAGCACCTGACCCTGAGCAAG GCCGACTACGAGAAGCACAAGGTGTACGCCTGCGAGGTGACCCAC CAGGGCCTGTCCAGCCCCGTGACCAAGAGCTTCAACCGGGGCGAG TGT
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[00197] Other antibodies of the invention include those where the amino acids or nucleic acids encoding the amino acids have been mutated, yet have at least 60, 70, 80, 90 or 95 percent identity to the sequences described in Table 1. In some embodiments, it include mutant amino acid sequences wherein no more than 1, 2, 3, 4 or 5 amino acids have been mutated in the variable regions when compared with the variable regions depicted in the sequence described in Table 1, while retaining substantially the same therapeutic activity.

[00198] Since each of these antibodies can bind to FGFR2/4, the VH, VL, full length light chain, and full length heavy chain sequences (amino acid sequences and the nucleotide sequences encoding the amino acid sequences) can be "mixed and matched" to create other FGFR2/4-binding antibodies of the invention. Such "mixed and matched" FGFR2/4-binding antibodies can be tested using the binding assays known in the art (*e.g.*, ELISAs, and other assays described in the Example section). When these chains are mixed and matched, a VH sequence from a particular VH/VL pairing should be replaced with a structurally similar VH sequence. Likewise a full length heavy chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length heavy chain sequence. Likewise, a VL sequence from a particular VH/VL pairing should be replaced with a structurally similar VL sequence. Likewise a full length light chain sequence from a particular full length heavy chain / full length light chain pairing should be replaced with a structurally similar full length light chain sequence. Accordingly, in one aspect, the invention provides an isolated monoclonal antibody or antigen binding region thereof having: a heavy chain variable region comprising an amino acid

sequence selected from the group consisting of SEQ ID NOs: 7, 27, 47, 67, 87 and 107; and a light chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 17, 37, 57, 77, 97, and 117; wherein the antibody specifically binds to FGFR2/4.

[00199] In another aspect, the invention provides (i) an isolated monoclonal antibody having: a full length heavy chain comprising an amino acid sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NOs: 9, 29, 49, 69, 89, and 109; and a full length light chain comprising an amino acid sequence that has been optimized for expression in the cell of a mammalian selected from the group consisting of SEQ ID NOs: 19, 39, 59, 79, 99, and 119; or (ii) a functional protein comprising an antigen binding portion thereof.

[00200] In another aspect, the present invention provides FGFR2/4-binding antibodies that comprise the heavy chain and light chain CDR1s, CDR2s and CDR3s as described in Table 1, or combinations thereof. The amino acid sequences of the VH CDR1s of the antibodies are shown in SEQ ID NOs: 1, 21, 41, 61, 81, and 101. The amino acid sequences of the VH CDR2s of the antibodies and are shown in SEQ ID NOs: 2, 22, 42, 62, 82, and 102. The amino acid sequences of the VH CDR3s of the antibodies are shown in SEQ ID NOs: 3, 23, 43, 63, 83, and 103. The amino acid sequences of the VL CDR1s of the antibodies are shown in SEQ ID NOs: 11, 31, 51, 71, 91, and 111. The amino acid sequences of the VL CDR2s of the antibodies are shown in SEQ ID NOs 12, 32, 52, 72, 92, and 112. The amino acid sequences of the VL CDR3s of the antibodies are shown in SEQ ID NOs: 13, 33, 53, 73, 93, and 113.

[00201] Given that each of these antibodies can bind to FGFR2/4 and that antigen-binding specificity is provided primarily by the CDR1, 2 and 3 regions, the VH CDR1, 2 and 3 sequences and VL CDR1, 2 and 3 sequences can be "mixed and matched" (i.e., CDRs from different antibodies can be mixed and match, although each antibody must contain a VH CDR1, 2 and 3 and a VL CDR1, 2 and 3 to create other C5-binding binding molecules of the invention. Such "mixed and matched" FGFR2/4-binding antibodies can be tested using the binding assays known in the art and those described in the Examples (*e.g.*, ELISAs). When VH CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VH sequence should be replaced with a structurally similar CDR sequence(s). Likewise, when VL CDR sequences are mixed and matched, the CDR1, CDR2 and/or CDR3 sequence from a particular VL sequence should be replaced with a structurally similar CDR sequence(s). It will be readily

apparent to the ordinarily skilled artisan that novel VH and VL sequences can be created by substituting one or more VH and/or VL CDR region sequences with structurally similar sequences from the CDR sequences shown herein for monoclonal antibodies of the present invention.

[00202] Accordingly, the present invention provides an isolated monoclonal antibody or antigen binding region thereof comprising a heavy chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 21, 41, 61, 81, and 101; a heavy chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 2, 22, 42, 62, 82, and 102; a heavy chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 3, 23, 43, 63, 83, and 103; a light chain CDR1 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 11, 31, 51, 71, 91, and 111; a light chain CDR2 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 12, 32, 52, 72, 92, and 112; and a light chain CDR3 comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 13, 33, 53, 73, 93, and 113; wherein the antibody specifically binds FGFR2/4.

[00203] In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to FGFR2/4 comprising a heavy chain CDR1 of SEQ ID NO:1, a heavy chain CDR2 of SEQ ID NO: 2; a heavy chain CDR3 of SEQ ID NO:3; a light chain CDR1 of SEQ ID NO:11; a light chain CDR2 of SEQ ID NO: 12; and a light chain CDR3 of SEQ ID NO: 13.

[00204] In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to FGFR2/4 comprising a heavy chain CDR1 of SEQ ID NO:21, a heavy chain CDR2 of SEQ ID NO: 22; a heavy chain CDR3 of SEQ ID NO:23; a light chain CDR1 of SEQ ID NO:31; a light chain CDR2 of SEQ ID NO: 32; and a light chain CDR3 of SEQ ID NO: 33.

[00205] In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to FGFR2/4 comprising a heavy chain CDR1 of SEQ ID NO:41, a heavy chain CDR2 of SEQ ID NO: 42; a heavy chain CDR3 of SEQ ID NO:43; a light chain CDR1 of SEQ ID NO:51; a light chain CDR2 of SEQ ID NO: 52; and a light chain CDR3 of SEQ ID NO: 53.

[00206] In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to FGFR2/4 comprising a heavy chain CDR1 of SEQ ID NO:61,

a heavy chain CDR2 of SEQ ID NO: 62; a heavy chain CDR3 of SEQ ID NO:63; a light chain CDR1 of SEQ ID NO:71; a light chain CDR2 of SEQ ID NO: 72; and a light chain CDR3 of SEQ ID NO: 73.

[00207] In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to FGFR2/4 comprising a heavy chain CDR1 of SEQ ID NO:81, a heavy chain CDR2 of SEQ ID NO: 82; a heavy chain CDR3 of SEQ ID NO:83; a light chain CDR1 of SEQ ID NO:91; a light chain CDR2 of SEQ ID NO: 92; and a light chain CDR3 of SEQ ID NO: 93.

[00208] In a specific embodiment, an antibody or antibody fragment (*e.g.*, antigen binding fragments) that specifically binds to FGFR2/4 comprising a heavy chain CDR1 of SEQ ID NO:101, a heavy chain CDR2 of SEQ ID NO: 102; a heavy chain CDR3 of SEQ ID NO:103; a light chain CDR1 of SEQ ID NO:111; a light chain CDR2 of SEQ ID NO: 112; and a light chain CDR3 of SEQ ID NO: 113.

[00209] In certain embodiments, an antibody that specifically binds to FGFR2/4 is an antibody or antibody fragment (*e.g.*, antigen binding fragment) that is described in Table 1.

1. Identification of Epitopes and Antibodies That Bind to the Same Epitope

[00210] In one embodiment, the present invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to an epitope on human FGFR2 comprising amino acids 176 (Lys) and 210 (Arg) of SEQ ID NO:137. In some aspects, the present invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind amino acids at positions 173 (Asn), 174 (Thr), 175 (Val), 176 (Lys), 178 (Arg), 208 (Lys), 209 (Val), 210 (Arg), 212 (Gln), 213 (His), 217 (Ile), 219 (Glu) of human FGFR2 as shown in SEQ ID NO:137.

[00211] In one embodiment, the present invention provides antibodies and antibody fragments (*e.g.*, antigen binding fragments) that bind to an epitope of amino acids 160-189 (KMEKRLHAVPAANTVKFRCPPAGGNPMTMR; SEQ ID NO: 136) and/or amino acids 198-216 (KMEKRLHAVPAANTVKFRC; SEQ ID NO: 141) of human FGFR2 (numbered according to P21802-3; SEQ ID NO: 137).

[00212] In one embodiment, the present invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to an epitope on human FGFR4 comprising amino 169 (Lys) and 203 (Arg) of SEQ ID NO:142. In some aspects, the present

invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind amino acids at positions 150 (Thr), 151 (His), 154 (Arg), 157 (Lys), 160 (His), 166 (Asn), 167 (Thr), 168 (Val), 169 (Lys), 171 (Arg), 173 (Pro), 174 (Ala), 201 (Arg), 202 (Leu), 203 (Arg), 204 (His), 205 (Gln), 206 (His), 207 (Trp), 210 (Val) and 212 (Glu) of human FGFR4 as shown in SEQ ID NO:142.

[00213] In one embodiment, the present invention provides antibodies and antibody fragments (*e.g.*, antigen binding fragments) that bind to an epitope of amino acids 150-174 (THPQRMEKKLHAVPAGNTVKFRCPA; SEQ ID NO: 132) and/or amino acids 201-212 (RLRHQHWSLVME; SEQ ID NO: 133) of human FGFR4 (numbered according to P22455-1; SEQ ID NO: 142).

[00214] In one embodiment, the present invention provides antibodies or antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to an epitope on human FGFR2 comprising amino acids 176 (Lys) and 210 (Arg) of SEQ ID NO:137 and to an epitope on human FGFR4 comprising amino acids 169 (Lys) and 203 (Arg) of SEQ ID NO:142.

[00215] In one embodiment, the present invention provides antibodies and antibody fragments (*e.g.*, antigen binding fragments) that bind to an epitope of amino acids 160-189 (KMEKRLHAVPAANTVKFRCPAGGNPMPMTMR; SEQ ID NO: 136) and amino acids 198-216 (KMEKRLHAVPAANTVKFRC; SEQ ID NO: 141) of human FGFR2 (numbered according to P21802-3; SEQ ID NO: 137) and an epitope of amino acids 150-174 (THPQRMEKKLHAVPAGNTVKFRCPA; SEQ ID NO: 132) and amino acids 201-212 (RLRHQHWSLVME; SEQ ID NO: 133) of human FGFR4 (numbered according to P22455-1; SEQ ID NO: 142).

[00216] The present invention also provides antibodies and antibody fragments (*e.g.*, antigen binding fragments) that specifically bind to the same epitope as do the anti-FGFR2/4 antibodies described in Table 1, or cross compete with the antibodies described in Table 1. Additional antibodies and antibody fragments (*e.g.*, antigen binding fragments) can therefore be identified based on their ability to cross-compete (*e.g.*, to competitively inhibit the binding of, in a statistically significant manner) with other antibodies of the invention in FGFR2 and/or FGFR4 binding assays. The ability of a test antibody to inhibit the binding of antibodies and antibody fragments (*e.g.*, antigen binding fragments) of the present invention to a FGFR2 and/or FGFR4 protein (*e.g.*, human FGFR2 and/or FGFR4) demonstrates that the test antibody can compete with

that antibody or antibody fragment (*e.g.*, antigen binding fragments) for binding to FGFR2 and/or FGFR4; such an antibody may, according to non-limiting theory, bind to the same or a related (*e.g.*, a structurally similar or spatially proximal) epitope on the FGFR2 and/or FGFR4 protein as the antibody or antibody fragment (*e.g.*, antigen binding fragments) with which it competes. In certain embodiments, the antibodies that bind to the same epitope on FGFR2 and/or FGFR4 as the antibodies or antibody fragments (*e.g.*, antigen binding fragments) described in Table 1 are human or humanized monoclonal antibodies. Such human or humanized monoclonal antibodies can be prepared and isolated as described herein.

2. Further Alteration of the Framework of Fc Region

[00217] The immunoconjugates of the invention may comprise modified antibodies or antigen binding fragments thereof that further comprise modifications to framework residues within VH and/or VL, *e.g.* to improve the properties of the antibody. In some embodiments, the framework modifications are made to decrease the immunogenicity of the antibody. For example, one approach is to "back-mutate" one or more framework residues to the corresponding germline sequence. More specifically, an antibody that has undergone somatic mutation may contain framework residues that differ from the germline sequence from which the antibody is derived. Such residues can be identified by comparing the antibody framework sequences to the germline sequences from which the antibody is derived. To return the framework region sequences to their germline configuration, the somatic mutations can be "back-mutated" to the germline sequence by, for example, site-directed mutagenesis. Such "back-mutated" antibodies are also intended to be encompassed by the invention.

[00218] Another type of framework modification involves mutating one or more residues within the framework region, or even within one or more CDR regions, to remove T-cell epitopes to thereby reduce the potential immunogenicity of the antibody. This approach is also referred to as "deimmunization" and is described in further detail in U.S. Patent Publication No. 20030153043 by Carr *et al.*

[00219] In addition or alternative to modifications made within the framework or CDR regions, antibodies of the invention may be engineered to include modifications within the Fc region, typically to alter one or more functional properties of the antibody, such as serum half-life, complement fixation, Fc receptor binding, and/or antigen-dependent cellular cytotoxicity. Furthermore, an antibody of the invention may be chemically modified (*e.g.*, one or more

chemical moieties can be attached to the antibody) or be modified to alter its glycosylation, again to alter one or more functional properties of the antibody. Each of these embodiments is described in further detail below.

[00220] In one embodiment, the hinge region of CH1 is modified such that the number of cysteine residues in the hinge region is altered, *e.g.*, increased or decreased. This approach is described further in U.S. Patent No. 5,677,425 by Bodmer *et al.* The number of cysteine residues in the hinge region of CH1 is altered to, for example, facilitate assembly of the light and heavy chains or to increase or decrease the stability of the antibody.

[00221] In another embodiment, the Fc hinge region of an antibody is mutated to decrease the biological half-life of the antibody. More specifically, one or more amino acid mutations are introduced into the CH2-CH3 domain interface region of the Fc-hinge fragment such that the antibody has impaired Staphylococcal protein A (SpA) binding relative to native Fc-hinge domain SpA binding. This approach is described in further detail in U.S. Patent No. 6,165,745 by Ward *et al.*

[00222] In yet other embodiments, the Fc region is altered by replacing at least one amino acid residue with a different amino acid residue to alter the effector functions of the antibody. For example, one or more amino acids can be replaced with a different amino acid residue such that the antibody has an altered affinity for an effector ligand but retains the antigen-binding ability of the parent antibody. The effector ligand to which affinity is altered can be, for example, an Fc receptor or the C1 component of complement. This approach is described in, *e.g.*, U.S. Patent Nos. 5,624,821 and 5,648,260, both by Winter *et al.*

[00223] In another embodiment, one or more amino acids selected from amino acid residues can be replaced with a different amino acid residue such that the antibody has altered C1q binding and/or reduced or abolished complement dependent cytotoxicity (CDC). This approach is described in, *e.g.*, U.S. Patent Nos. 6,194,551 by Idusogie *et al.*

[00224] In another embodiment, one or more amino acid residues are altered to thereby alter the ability of the antibody to fix complement. This approach is described in, *e.g.*, the PCT Publication WO 94/29351 by Bodmer *et al.* In a specific embodiment, one or more amino acids of an antibody or antigen binding fragment thereof of the present invention are replaced by one or more allotypic amino acid residues, such as those shown in FIG. 4 for the IgG1 subclass and the kappa isotype. Allotypic amino acid residues also include, but are not limited to, the constant

region of the heavy chain of the IgG1, IgG2, and IgG3 subclasses as well as the constant region of the light chain of the kappa isotype as described by Jefferis *et al.*, MAbs. 1:332-338 (2009).

[00225] In yet another embodiment, the Fc region is modified to increase the ability of the antibody to mediate antibody dependent cellular cytotoxicity (ADCC) and/or to increase the affinity of the antibody for an Fcγ receptor by modifying one or more amino acids. This approach is described in, *e.g.*, the PCT Publication WO 00/42072 by Presta. Moreover, the binding sites on human IgG1 for FcγR1, FcγR2, FcγR3 and FcRn have been mapped and variants with improved binding have been described (see Shields *et al.*, J. Biol. Chem. 276:6591-6604, 2001).

[00226] In still another embodiment, the glycosylation of an antibody is modified. For example, an aglycosylated antibody can be made (*i.e.*, the antibody lacks glycosylation). Glycosylation can be altered to, for example, increase the affinity of the antibody for "antigen." Such carbohydrate modifications can be accomplished by, for example, altering one or more sites of glycosylation within the antibody sequence. For example, one or more amino acid substitutions can be made that result in elimination of one or more variable region framework glycosylation sites to thereby eliminate glycosylation at that site. Such aglycosylation may increase the affinity of the antibody for antigen. Such an approach is described in, *e.g.*, U.S. Patent Nos. 5,714,350 and 6,350,861 by Co *et al.*

[00227] Additionally or alternatively, an antibody can be made that has an altered type of glycosylation, such as a hypofucosylated antibody having reduced amounts of fucosyl residues or an antibody having increased bisecting GlcNAc structures. Such altered glycosylation patterns have been demonstrated to increase the ADCC ability of antibodies. Such carbohydrate modifications can be accomplished by, for example, expressing the antibody in a host cell with altered glycosylation machinery. Cells with altered glycosylation machinery have been described in the art and can be used as host cells in which to express recombinant antibodies of the invention to thereby produce an antibody with altered glycosylation. For example, EP 1,176,195 by Hang *et al.* describes a cell line with a functionally disrupted FUT8 gene, which encodes a fucosyl transferase, such that antibodies expressed in such a cell line exhibit hypofucosylation. PCT Publication WO 03/035835 by Presta describes a variant CHO cell line, Lec13 cells, with reduced ability to attach fucose to Asn(297)-linked carbohydrates, also resulting in hypofucosylation of antibodies expressed in that host cell (see also Shields *et al.*, (2002) J. Biol. Chem. 277:26733-26740). PCT Publication WO 99/54342 by Umana *et al.* describes cell lines engineered to

express glycoprotein-modifying glycosyl transferases (*e.g.*, beta(1,4)-N acetylglucosaminyltransferase III (GnTIII)) such that antibodies expressed in the engineered cell lines exhibit increased bisecting GlcNac structures which results in increased ADCC activity of the antibodies (see also Umana *et al.*, Nat. Biotech. 17:176-180, 1999).

[00228] In another embodiment, the antibody is modified to increase its biological half-life. Various approaches are possible. For example, one or more of the following mutations can be introduced: T252L, T254S, T256F, as described in U.S. Patent No. 6,277,375 to Ward. Alternatively, to increase the biological half-life, the antibody can be altered within the CH1 or CL region to contain a salvage receptor binding epitope taken from two loops of a CH2 domain of an Fc region of an IgG, as described in U.S. Patent Nos. 5,869,046 and 6,121,022 by Presta *et al.*

3. Production of the FGFR2/4 Antibodies

[00229] Anti-FGFR2/4 antibodies and antibody fragments (*e.g.*, antigen binding fragments) thereof can be produced by any means known in the art, including but not limited to, recombinant expression, chemical synthesis, and enzymatic digestion of antibody tetramers, whereas full-length monoclonal antibodies can be obtained by, *e.g.*, hybridoma or recombinant production. Recombinant expression can be from any appropriate host cells known in the art, for example, mammalian host cells, bacterial host cells, yeast host cells, insect host cells, etc.

[00230] The invention further provides polynucleotides encoding the antibodies described herein, *e.g.*, polynucleotides encoding heavy or light chain variable regions or segments comprising the complementarity determining regions as described herein. In some embodiments, the polynucleotide encoding the heavy chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide selected from the group consisting of SEQ ID NOs: 8, 28, 48, 68, 88, and 108. In some embodiments, the polynucleotide encoding the light chain variable regions has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide selected from the group consisting of SEQ ID NOs: 18, 38, 58, 78, 98, and 118.

[00231] In some embodiments, the polynucleotide encoding the heavy chain has at least 85%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 10, 30, 50, 70, 90, or 110. In some embodiments, the polynucleotide encoding the light chain has at least 85%, 89%, 90%, 91%, 92%,

93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100% nucleic acid sequence identity with a polynucleotide of SEQ ID NO: 20, 40, 60, 80, 100, or 120.

[00232] The polynucleotides of the invention can encode only the variable region sequence of an anti-FGFR2/4 antibody. They can also encode both a variable region and a constant region of the antibody. Some of the polynucleotide sequences encode a polypeptide that comprises variable regions of both the heavy chain and the light chain of one of the exemplified mouse anti-FGFR2 and/or FGFR4 antibody. Some other polynucleotides encode two polypeptide segments that respectively are substantially identical to the variable regions of the heavy chain and the light chain of one of the mouse antibodies.

[00233] The polynucleotide sequences can be produced by de novo solid-phase DNA synthesis or by PCR mutagenesis of an existing sequence (*e.g.*, sequences as described in the Examples below) encoding an anti-FGFR2/4 antibody or its binding fragment. Direct chemical synthesis of nucleic acids can be accomplished by methods known in the art, such as the phosphotriester method of Narang *et al.*, Meth. Enzymol. 68:90, 1979; the phosphodiester method of Brown *et al.*, Meth. Enzymol. 68:109, 1979; the diethylphosphoramidite method of Beaucage *et al.*, Tetra. Lett., 22:1859, 1981; and the solid support method of U.S. Patent No. 4,458,066. Introducing mutations to a polynucleotide sequence by PCR can be performed as described in, *e.g.*, PCR Technology: Principles and Applications for DNA Amplification, H.A. Erlich (Ed.), Freeman Press, NY, NY, 1992; PCR Protocols: A Guide to Methods and Applications, Innis *et al.* (Ed.), Academic Press, San Diego, CA, 1990; Mattila *et al.*, Nucleic Acids Res. 19:967, 1991; and Eckert *et al.*, PCR Methods and Applications 1:17, 1991.

[00234] Also provided in the invention are expression vectors and host cells for producing the anti-FGFR2/4 antibodies described above. Various expression vectors can be employed to express the polynucleotides encoding the anti-FGFR2/4 antibody chains or binding fragments. Both viral-based and nonviral expression vectors can be used to produce the antibodies in a mammalian host cell. Nonviral vectors and systems include plasmids, episomal vectors, typically with an expression cassette for expressing a protein or RNA, and human artificial chromosomes (see, *e.g.*, Harrington *et al.*, Nat Genet 15:345, 1997). For example, nonviral vectors useful for expression of the anti-FGFR2/4 polynucleotides and polypeptides in mammalian (*e.g.*, human) cells include pThioHis A, B & C, pcDNATM3.1/His, pEBVHis A, B & C (Invitrogen, San Diego, CA), MPSV vectors, and numerous other vectors known in the art for expressing other proteins.

Useful viral vectors include vectors based on retroviruses, adenoviruses, adenoassociated viruses, herpes viruses, vectors based on SV40, papilloma virus, HBP Epstein Barr virus, vaccinia virus vectors and Semliki Forest virus (SFV). See, Brent *et al.*, supra; Smith, Annu. Rev. Microbiol. 49:807, 1995; and Rosenfeld *et al.*, Cell 68:143, 1992.

[00235] The choice of expression vector depends on the intended host cells in which the vector is to be expressed. Typically, the expression vectors contain a promoter and other regulatory sequences (*e.g.*, enhancers) that are operably linked to the polynucleotides encoding an anti-FGFR2/4 antibody chain or fragment. In some embodiments, an inducible promoter is employed to prevent expression of inserted sequences except under inducing conditions. Inducible promoters include, *e.g.*, arabinose, lacZ, metallothionein promoter or a heat shock promoter. Cultures of transformed organisms can be expanded under noninducing conditions without biasing the population for coding sequences whose expression products are better tolerated by the host cells. In addition to promoters, other regulatory elements may also be required or desired for efficient expression of an anti-FGFR2/4 antibody chain or fragment. These elements typically include an ATG initiation codon and adjacent ribosome binding site or other sequences. In addition, the efficiency of expression may be enhanced by the inclusion of enhancers appropriate to the cell system in use (see, *e.g.*, Scharf *et al.*, Results Probl. Cell Differ. 20:125, 1994; and Bittner *et al.*, Meth. Enzymol., 153:516, 1987). For example, the SV40 enhancer or CMV enhancer may be used to increase expression in mammalian host cells.

[00236] The expression vectors may also provide a secretion signal sequence position to form a fusion protein with polypeptides encoded by inserted anti-FGFR2/4 antibody sequences. More often, the inserted anti-FGFR2/4 antibody sequences are linked to a signal sequences before inclusion in the vector. Vectors to be used to receive sequences encoding anti-FGFR2/4 antibody light and heavy chain variable domains sometimes also encode constant regions or parts thereof. Such vectors allow expression of the variable regions as fusion proteins with the constant regions thereby leading to production of intact antibodies or fragments thereof. Typically, such constant regions are human.

[00237] The host cells for harboring and expressing the anti-FGFR2/4 antibody chains can be either prokaryotic or eukaryotic. *E. coli* is one prokaryotic host useful for cloning and expressing the polynucleotides of the present invention. Other microbial hosts suitable for use include bacilli, such as *Bacillus subtilis*, and other enterobacteriaceae, such as *Salmonella*,

Serratia, and various Pseudomonas species. In these prokaryotic hosts, one can also make expression vectors, which typically contain expression control sequences compatible with the host cell (*e.g.*, an origin of replication). In addition, any number of a variety of well-known promoters will be present, such as the lactose promoter system, a tryptophan (*trp*) promoter system, a beta-lactamase promoter system, or a promoter system from phage lambda. The promoters typically control expression, optionally with an operator sequence, and have ribosome binding site sequences and the like, for initiating and completing transcription and translation. Other microbes, such as yeast, can also be employed to express anti-FGFR2/4 polypeptides of the invention. Insect cells in combination with baculovirus vectors can also be used.

[00238] In some preferred embodiments, mammalian host cells are used to express and produce the anti-FGFR2/4 polypeptides of the present invention. For example, they can be either a hybridoma cell line expressing endogenous immunoglobulin genes (*e.g.*, the myeloma hybridoma clones as described in the Examples) or a mammalian cell line harboring an exogenous expression vector (*e.g.*, the SP2/0 myeloma cells exemplified below). These include any normal mortal or normal or abnormal immortal animal or human cell. For example, a number of suitable host cell lines capable of secreting intact immunoglobulins have been developed, including the CHO cell lines, various Cos cell lines, HeLa cells, myeloma cell lines, transformed B-cells and hybridomas. The use of mammalian tissue cell culture to express polypeptides is discussed generally in, *e.g.*, Winnacker, From Genes to Clones, VCH Publishers, N.Y., N.Y., 1987. Expression vectors for mammalian host cells can include expression control sequences, such as an origin of replication, a promoter, and an enhancer (see, *e.g.*, Queen *et al.*, Immunol. Rev. 89:49-68, 1986), and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. These expression vectors usually contain promoters derived from mammalian genes or from mammalian viruses. Suitable promoters may be constitutive, cell type-specific, stage-specific, and/or modulatable or regulatable. Useful promoters include, but are not limited to, the metallothionein promoter, the constitutive adenovirus major late promoter, the dexamethasone-inducible MMTV promoter, the SV40 promoter, the MRP polIII promoter, the constitutive MPSV promoter, the tetracycline-inducible CMV promoter (such as the human immediate-early CMV promoter), the constitutive CMV promoter, and promoter-enhancer combinations known in the art.

[00239] Methods for introducing expression vectors containing the polynucleotide sequences of interest vary depending on the type of cellular host. For example, calcium chloride transfection is commonly utilized for prokaryotic cells, whereas calcium phosphate treatment or electroporation may be used for other cellular hosts (see generally Sambrook *et al.*, supra). Other methods include, *e.g.*, electroporation, calcium phosphate treatment, liposome-mediated transformation, injection and microinjection, ballistic methods, virosomes, immunoliposomes, polycation:nucleic acid conjugates, naked DNA, artificial virions, fusion to the herpes virus structural protein VP22 (Elliot and O'Hare, Cell 88:223, 1997), agent-enhanced uptake of DNA, and *ex vivo* transduction. For long-term, high-yield production of recombinant proteins, stable expression will often be desired. For example, cell lines which stably express anti-FGFR2 and/or FGFR4 antibody chains or binding fragments can be prepared using expression vectors of the invention which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth of cells which successfully express the introduced sequences in selective media. Resistant, stably transfected cells can be proliferated using tissue culture techniques appropriate to the cell type.

Therapeutic and Diagnostic Uses

[00240] The antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates of the invention are useful in a variety of applications including, but not limited to, treatment of cancer, such as solid cancers. In certain embodiments, the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates of the invention are useful for inhibiting tumor growth, inducing differentiation, reducing tumor volume, and/or reducing the tumorigenicity of a tumor. The methods of use can be *in vitro*, *ex vivo*, or *in vivo* methods.

[00241] In one aspect, the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates of the invention are useful for detecting the presence of FGFR2 or FGFR4 in a biological sample. The term "detecting" as used herein encompasses quantitative or qualitative detection. In certain embodiments, a biological sample comprises a cell or tissue. In

certain embodiments, such tissues include normal and/or cancerous tissues that express FGFR2 or FGFR4 at higher levels relative to other tissues.

[00242] In one aspect, the invention provides a method of detecting the presence of FGFR2 or FGFR4 in a biological sample. In certain embodiments, the method comprises contacting the biological sample with an anti-FGFR2/4 antibody under conditions permissive for binding of the antibody to the antigen, and detecting whether a complex is formed between the antibody and the antigen.

[00243] In one aspect, the invention provides a method of diagnosing a disorder associated with increased expression of FGFR2 or FGFR4. In certain embodiments, the method comprises contacting a test cell with an anti-FGFR2/4 antibody; determining the level of expression (either quantitatively or qualitatively) of FGFR2 or FGFR4 on the test cell by detecting binding of the anti-FGFR2/4 antibody to the FGFR2 or FGFR4 antigen; and comparing the level of expression of FGFR2 or FGFR4 in the test cell with the level of expression of FGFR2 or FGFR4 on a control cell (*e.g.*, a normal cell of the same tissue origin as the test cell or a cell that expresses FGFR2 or FGFR4 at levels comparable to such a normal cell), wherein a higher level of expression of FGFR2 or FGFR4 on the test cell as compared to the control cell indicates the presence of a disorder associated with increased expression of FGFR2 or FGFR4. In certain embodiments, the test cell is obtained from an individual suspected of having a disorder associated with increased expression of FGFR2 or FGFR4. In certain embodiments, the disorder is a cell proliferative disorder, such as a cancer or a tumor. In certain embodiments, the method comprises measuring the copy number of the FGFR2 gene in a test cell. In certain embodiments, the method comprises detecting a PAX-FOXO translocation mutation. Copy number of a gene and/or translocation mutations can be detected using standard methods known in the art, for example, PCR, RTPCR, etc..

[00244] In certain embodiments, a method of diagnosis or detection, such as those described above, comprises detecting binding of an anti-FGFR2/4 antibody to FGFR2 or FGFR4 expressed on the surface of a cell or in a membrane preparation obtained from a cell expressing FGFR2 or FGFR4 on its surface. An exemplary assay for detecting binding of an anti-FGFR2/4 antibody to FGFR2 or FGFR4 expressed on the surface of a cell is a "FACS" assay.

[00245] Certain other methods can be used to detect binding of anti-FGFR2/4 antibodies to FGFR2 or FGFR4. Such methods include, but are not limited to, antigen-binding assays that are

well known in the art, such as western blots, radioimmunoassays, ELISA (enzyme linked immunosorbent assay), "sandwich" immunoassays, immunoprecipitation assays, fluorescent immunoassays, protein A immunoassays, and immunohistochemistry (IHC).

[00246] In certain embodiments, anti-FGFR2/4 antibodies are labeled. Labels include, but are not limited to, labels or moieties that are detected directly (such as fluorescent, chromophoric, electron-dense, chemiluminescent, and radioactive labels), as well as moieties, such as enzymes or ligands, that are detected indirectly, *e.g.*, through an enzymatic reaction or molecular interaction.

[00247] In certain embodiments, anti-FGFR2/4 antibodies are immobilized on an insoluble matrix. Immobilization entails separating the anti-FGFR2/4 antibody from any FGFR2 or FGFR4 proteins that remains free in solution. This conventionally is accomplished by either insolubilizing the anti-FGFR2/4 antibody before the assay procedure, as by adsorption to a water-insoluble matrix or surface (Bennich et al, U.S. Patent No. 3,720,760), or by covalent coupling (for example, using glutaraldehyde cross-linking), or by insolubilizing the anti-FGFR2/4 antibody after formation of a complex between the anti-FGFR2/4 antibody and FGFR2 or FGFR4 protein, *e.g.*, by immunoprecipitation.

[00248] Any of the above embodiments of diagnosis or detection can be carried out using an immunoconjugate of the invention in place of or in addition to an anti-FGFR2/4 antibody.

[00249] In one embodiment, the invention provides a method of treating, or preventing a disease comprising administering the antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates of the invention to a patient. In certain embodiments, the disease treated with the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates of the invention is a cancer. Examples of diseases which can be treated and/or prevented include, but are not limited to, adrenocortical carcinoma, bladder cancer, bone cancer, breast cancer, central nervous system atypical teratoid/rhabdoid tumors, colon cancer, colorectal cancer, embryonal tumors, endometrial cancer, gastric cancer, head and neck cancer, hepatocellular cancer, Kaposi sarcoma, liver cancer, non-small cell lung cancer, rectal cancer, rhabdomyosarcoma, small cell lung cancer, small intestine cancer, soft tissue sarcoma, squamous cell carcinoma, squamous neck cancer, stomach cancer, uterine cancer, vaginal cancer, vulvar cancer. In certain embodiments, the cancer is characterized by FGFR2 or FGFR4 expressing cells to which the antibodies, antibody fragments (*e.g.*, antigen binding fragments), and antibody drug conjugates of the invention binds. In certain embodiments, the cancer is characterize by an

increase in DNA copy number of FGFR2 or FGFR4. In certain embodiments, the cancer is characterized by an increase in expression of FGFR2 or FGFR4, for example, as measured by an increase in FGFR2 or FGFR4 RNA, respectively. The present invention provides for methods of treating cancer comprising administering a therapeutically effective amount of the antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates of the invention. In certain embodiments, the cancer is a solid cancer. In certain embodiments, the subject is a human. In certain embodiments, the cancer is a resistant cancer and/or relapsed cancer. In certain aspects, for example, the resistant cancer is resistant to tyrosine kinase inhibitors, including but not limited to, EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGFR inhibitors and Met inhibitors. In certain embodiments the cancer is a Her2 resistant cancer.

[00250] In certain embodiments, the invention provides for methods of inhibiting tumor growth comprising administering to a subject a therapeutically effective amount of the antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates of the invention. In certain embodiments, the subject is a human. In certain embodiments, the subject has a tumor or has had a tumor removed. In certain embodiments, the tumor is resistant to other tyrosine kinase inhibitors, including but not limited to, EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGFR inhibitors and Met inhibitors.

[00251] In certain embodiments, the tumor expresses the FGFR2 or FGFR4 to which the anti-FGFR2/4 antibody binds. In certain embodiments, the tumor overexpresses the human FGFR2 or FGFR4. In certain embodiments, the tumor has an increase copy number of the FGFR2 gene. In certain embodiments, the tumor has a PAX-FOXO translocation mutation causing overexpression of FGFR4.

[00252] The present invention also provides for methods of selecting patients for treatment with antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates of the invention comprising administering a therapeutically effective amount of said antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates. In certain aspects the method comprises selecting patients with a tyrosine kinase inhibitor resistant cancer. In certain aspects it is contemplated that the tyrosine kinase inhibitor resistant cancer is resistant to EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGFR inhibitors and/or Met inhibitors. In certain aspects it is contemplated that the resistant cancer is a Her2 resistant cancer. More specifically it is contemplated that the Her2 resistant cancer does not respond to trastuzumab or

trastuzumab emtansine. In certain aspects it is contemplated that the cancer is a de novo resistant cancer, and in still other aspects it is contemplated that the cancer is a relapsed cancer, for example a Her2 relapsed cancer. In certain aspects of the invention the methods comprise selecting a patient with a de novo resistant or relapsed cancer and measuring for expression of FGFR2 and/or FGFR4. It is contemplated that in certain aspects the relapsed cancer or tumor was not initially an FGFR2 or FGFR4 expressing cancer or tumor, but becomes an FGFR2 and/or FGFR4 positive cancer that is a tyrosine kinase resistant or relapsed cancer or tumor after treatment with tyrosine kinase inhibitors (for example, trastuzumab or trastuzumab emtansine).

[00253] For the treatment of the disease, the appropriate dosage of the antibodies, antibody fragments (*e.g.*, antigen binding fragments), or antibody drug conjugates of the present invention depends on various factors, such as the type of disease to be treated, the severity and course of the disease, the responsiveness of the disease, previous therapy, patient's clinical history, and so on. The antibody or agent can be administered one time or over a series of treatments lasting from several days to several months, or until a cure is effected or a diminution of the disease state is achieved (*e.g.*, reduction in tumor size). Optimal dosing schedules can be calculated from measurements of drug accumulation in the body of the patient and will vary depending on the relative potency of an individual antibody, antibody fragment (*e.g.*, antigen binding fragment), or antibody drug conjugates. In certain embodiments, dosage is from 0.01mg to 10 mg (*e.g.*, 0.01 mg, 0.05mg, 0.1mg, 0.5mg, 1mg, 2mg, 3mg, 4mg, 5mg, 7mg, 8mg, 9mg, or 10mg) per kg of body weight, and can be given once or more daily, weekly, monthly or yearly. In certain embodiments, the antibody, antibody fragment (*e.g.*, antigen binding fragment), or antibody drug conjugate of the present invention is given once every two weeks or once every three weeks. The treating physician can estimate repetition rates for dosing based on measured residence times and concentrations of the drug in bodily fluids or tissues.

Combination Therapy

[00254] In certain instances, an antibody, antibody fragment (*e.g.*, antigen binding fragment), or antibody drug conjugate of the present invention is combined with other therapeutic agents, such as other anti-cancer agents, anti-allergic agents, anti-nausea agents (or anti-emetics), pain relievers, cytoprotective agents, and combinations thereof.

[00255] General Chemotherapeutic agents considered for use in combination therapies include anastrozole (Arimidex[®]), bicalutamide (Casodex[®]), bleomycin sulfate (Blenoxane[®]),

busulfan (Myleran[®]), busulfan injection (Busulfex[®]), capecitabine (Xeloda[®]), N4-pentoxycarbonyl-5-deoxy-5-fluorocytidine, carboplatin (Paraplatin[®]), carmustine (BiCNU[®]), chlorambucil (Leukeran[®]), cisplatin (Platinol[®]), cladribine (Leustatin[®]), cyclophosphamide (Cytosan[®] or Neosar[®]), cytarabine, cytosine arabinoside (Cytosar-U[®]), cytarabine liposome injection (DepoCyt[®]), dacarbazine (DTIC-Dome[®]), dactinomycin (Actinomycin D, Cosmegen), daunorubicin hydrochloride (Cerubidine[®]), daunorubicin citrate liposome injection (DaunoXome[®]), dexamethasone, docetaxel (Taxotere[®]), doxorubicin hydrochloride (Adriamycin[®], Rubex[®]), etoposide (Vepesid[®]), fludarabine phosphate (Fludara[®]), 5-fluorouracil (Adrucil[®], Efudex[®]), flutamide (Eulexin[®]), tezacitibine, Gemcitabine (difluorodeoxycytidine), hydroxyurea (Hydrea[®]), Idarubicin (Idamycin[®]), ifosfamide (IFEX[®]), irinotecan (Camptosar[®]), L-asparaginase (ELSPAR[®]), leucovorin calcium, melphalan (Alkeran[®]), 6-mercaptopurine (Purinethol[®]), methotrexate (Folex[®]), mitoxantrone (Novantrone[®]), mylotarg, paclitaxel (Taxol[®]), phoenix (Yttrium90/MX-DTPA), pentostatin, polifeprosan 20 with carmustine implant (Gliadel[®]), tamoxifen citrate (Nolvadex[®]), teniposide (Vumon[®]), 6-thioguanine, thiotepa, tirapazamine (Tirazone[®]), topotecan hydrochloride for injection (Hycamptin[®]), vinblastine (Velban[®]), vincristine (Oncovin[®]), and vinorelbine (Navelbine[®]).

[00256] In one embodiment, an antibody, antibody fragment (*e.g.*, antigen binding fragment), or antibody drug conjugate of the present invention is combined in a pharmaceutical combination formulation, or dosing regimen as combination therapy, with a second compound having anti-cancer properties. The second compound of the pharmaceutical combination formulation or dosing regimen can have complementary activities to the antibody or immunoconjugate of the combination such that they do not adversely affect each other. For example, an antibody, antibody fragment (*e.g.*, antigen binding fragment), or antibody drug conjugate of the present invention can be administered in combination with, but not limited to, a chemotherapeutic agent, a tyrosine kinase inhibitor, a FGF downstream signaling pathway inhibitor, IAP inhibitors, Bcl2 inhibitors, Mcl1 inhibitors, and other FGFR2 inhibitors.

[00257] The term “pharmaceutical combination” as used herein refers to either a fixed combination in one dosage unit form, or non-fixed combination or a kit of parts for the combined administration where two or more therapeutic agents may be administered independently at the

same time or separately within time intervals, especially where these time intervals allow that the combination partners show a cooperative, *e.g.* synergistic effect.

[00258] The term "combination therapy" refers to the administration of two or more therapeutic agents to treat a therapeutic condition or disorder described in the present disclosure. Such administration encompasses co-administration of these therapeutic agents in a substantially simultaneous manner, such as in a single capsule having a fixed ratio of active ingredients. Alternatively, such administration encompasses co-administration in multiple, or in separate containers (*e.g.*, capsules, powders, and liquids) for each active ingredient. Powders and/or liquids may be reconstituted or diluted to a desired dose prior to administration. In addition, such administration also encompasses use of each type of therapeutic agent in a sequential manner, either at approximately the same time or at different times. In either case, the treatment regimen will provide beneficial effects of the drug combination in treating the conditions or disorders described herein.

[00259] The combination therapy can provide "synergy" and prove "synergistic", *i.e.*, the effect achieved when the active ingredients used together is greater than the sum of the effects that results from using the compounds separately. A synergistic effect can be attained when the active ingredients are: (1) co-formulated and administered or delivered simultaneously in a combined, unit dosage formulation; (2) delivered by alternation or in parallel as separate formulations; or (3) by some other regimen. When delivered in alternation therapy, a synergistic effect can be attained when the compounds are administered or delivered sequentially, *e.g.*, by different injections in separate syringes. In general, during alternation therapy, an effective dosage of each active ingredient is administered sequentially, *i.e.*, serially, whereas in combination therapy, effective dosages of two or more active ingredients are administered together.

[00260] In one aspect, the present invention provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate of the present invention in combination with one or more tyrosine kinase inhibitors, including but not limited to, EGFR inhibitors, Her2 inhibitors, Her3 inhibitors, IGFR inhibitors, and Met inhibitors.

[00261] For example, tyrosine kinase inhibitors include but are not limited to, Erlotinib hydrochloride (Tarceva®); Linifanib (N-[4-(3-amino-1H-indazol-4-yl)phenyl]-N'-(2-fluoro-5-methylphenyl)urea, also known as ABT 869, available from Genentech); Sunitinib malate (Sutent®); Bosutinib (4-[(2,4-dichloro-5-methoxyphenyl)amino]-6-methoxy-7-[3-(4-

methylpiperazin-1-yl)propoxy]quinoline-3-carbonitrile, also known as SKI-606, and described in US Patent No. 6,780,996); Dasatinib (Sprycel®); Pazopanib (Votrient®); Sorafenib (Nexavar®); Zactima (ZD6474); and Imatinib or Imatinib mesylate (Gilevec® and Gleevec®).

[00262] Epidermal growth factor receptor (EGFR) inhibitors include but are not limited to, Erlotinib hydrochloride (Tarceva®), Gefitinib (Iressa®); N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[[(3"S")-tetrahydro-3-furanyl]oxy]-6-quinazoliny]-4(dimethylamino)-2-butenamide, Tovok®); Vandetanib (Caprelsa®); Lapatinib (Tykerb®); (3R,4R)-4-Amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); Canertinib dihydrochloride (CI-1033); 6-[4-[(4-Ethyl-1-piperaziny)methyl]phenyl]-N-[(1R)-1-phenylethyl]-7H-Pyrrolo[2,3-d]pyrimidin-4-amine (AEE788, CAS 497839-62-0); Mubritinib (TAK165); Pelitinib (EKB569); Afatinib (BIBW2992); Neratinib (HKI-272); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS599626); N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[[(3 α ,5 β ,6 α)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8); and 4-[4-[(1R)-1-Phenylethyl]amino]-7H-pyrrolo[2,3-d]pyrimidin-6-yl]-phenol (PKI166, CAS 187724-61-4).

[00263] EGFR antibodies include but are not limited to, Cetuximab (Erbix®); Panitumumab (Vectibix®); Matuzumab (EMD-72000); Trastuzumab (Herceptin®); Nimotuzumab (hR3); Zalutumumab; TheraCIM h-R3; MDX0447 (CAS 339151-96-1); and ch806 (mAb-806, CAS 946414-09-1).

[00264] Human Epidermal Growth Factor Receptor 2 (Her2 receptor) (also known as Neu, ErbB-2, CD340, or p185) inhibitors include but are not limited to, Trastuzumab (Herceptin®); Pertuzumab (Omnitarg®); trastuzumab emtansine (Kadcyla®); Neratinib (HKI-272, (2E)-N-[4-[[3-chloro-4-[(pyridin-2-yl)methoxy]phenyl]amino]-3-cyano-7-ethoxyquinolin-6-yl]-4-(dimethylamino)but-2-enamide, and described PCT Publication No. WO 05/028443); Lapatinib or Lapatinib ditosylate (Tykerb®); (3R,4R)-4-amino-1-((4-((3-methoxyphenyl)amino)pyrrolo[2,1-f][1,2,4]triazin-5-yl)methyl)piperidin-3-ol (BMS690514); (2E)-N-[4-[(3-Chloro-4-fluorophenyl)amino]-7-[[[(3S)-tetrahydro-3-furanyl]oxy]-6-quinazoliny]-4-(dimethylamino)-2-butenamide (BIBW-2992, CAS 850140-72-6); N-[4-[[1-[(3-Fluorophenyl)methyl]-1H-indazol-5-yl]amino]-5-methylpyrrolo[2,1-f][1,2,4]triazin-6-yl]-carbamic acid, (3S)-3-morpholinylmethyl ester (BMS 599626, CAS 714971-09-2); Canertinib

dihydrochloride (PD183805 or CI-1033); and N-(3,4-Dichloro-2-fluorophenyl)-6-methoxy-7-[[[(3 α ,5 β ,6 α)-octahydro-2-methylcyclopenta[c]pyrrol-5-yl]methoxy]-4-quinazolinamine (XL647, CAS 781613-23-8).

[00265] Her3 inhibitors include but are not limited to, LJM716, MM-121, AMG-888, RG7116, REGN-1400, AV-203, MP-RM-1, MM-111, and MEHD-7945A.

[00266] MET inhibitors include but are not limited to, Cabozantinib (XL184, CAS 849217-68-1); Foretinib (GSK1363089, formerly XL880, CAS 849217-64-7); Tivantinib (ARQ197, CAS 1000873-98-2); 1-(2-Hydroxy-2-methylpropyl)-N-(5-(7-methoxyquinolin-4-yloxy)pyridin-2-yl)-5-methyl-3-oxo-2-phenyl-2,3-dihydro-1*H*-pyrazole-4-carboxamide (AMG 458); Cryzotinib (Xalkori®, PF-02341066); (3*Z*)-5-(2,3-Dihydro-1*H*-indol-1-ylsulfonyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1*H*-pyrrol-2-yl}methylene)-1,3-dihydro-2*H*-indol-2-one (SU11271); (3*Z*)-N-(3-Chlorophenyl)-3-({3,5-dimethyl-4-[(4-methylpiperazin-1-yl)carbonyl]-1*H*-pyrrol-2-yl}methylene)-N-methyl-2-oxoindoline-5-sulfonamide (SU11274); (3*Z*)-N-(3-Chlorophenyl)-3-{{3,5-dimethyl-4-(3-morpholin-4-ylpropyl)-1*H*-pyrrol-2-yl}methylene}-N-methyl-2-oxoindoline-5-sulfonamide (SU11606); 6-[Difluoro[6-(1-methyl-1*H*-pyrazol-4-yl)-1,2,4-triazolo[4,3-*b*]pyridazin-3-yl]methyl]-quinoline (JNJ38877605, CAS 943540-75-8); 2-[4-[1-(Quinolin-6-ylmethyl)-1*H*-[1,2,3]triazolo[4,5-*b*]pyrazin-6-yl]-1*H*-pyrazol-1-yl]ethanol (PF04217903, CAS 956905-27-4); N-((2*R*)-1,4-Dioxan-2-ylmethyl)-N-methyl-N'-[3-(1-methyl-1*H*-pyrazol-4-yl)-5-oxo-5*H*-benzo[4,5]cyclohepta[1,2-*b*]pyridin-7-yl]sulfamide (MK2461, CAS 917879-39-1); 6-[[6-(1-Methyl-1*H*-pyrazol-4-yl)-1,2,4-triazolo[4,3-*b*]pyridazin-3-yl]thio]-quinoline (SGX523, CAS 1022150-57-7); and (3*Z*)-5-[[[(2,6-Dichlorophenyl)methyl]sulfonyl]-3-[[3,5-dimethyl-4-[[[(2*R*)-2-(1-pyrrolidinylmethyl)-1-pyrrolidinyl]carbonyl]-1*H*-pyrrol-2-yl]methylene]-1,3-dihydro-2*H*-indol-2-one (PHA665752, CAS 477575-56-7).

[00267] IGF1R inhibitors include but are not limited to, BMS-754807, XL-228, OSI-906, GSK0904529A, A-928605, AXL1717, KW-2450, MK0646, AMG479, IMCA12, MEDI-573, and BI836845. See *e.g.*, Yee, JNCI, 104; 975 (2012) for review.

[00268] In another aspect, the present invention provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate of the present invention in combination with one or more FGF downstream signaling pathway inhibitors, including but not limited to, MEK inhibitors, Braf inhibitors, PI3K/Akt inhibitors, SHP2 inhibitors, and also mTor.

[00269] For example, mitogen-activated protein kinase (MEK) inhibitors include but are not limited to, XL-518 (also known as GDC-0973, Cas No. 1029872-29-4, available from ACC Corp.); 2-[(2-Chloro-4-iodophenyl)amino]-N-(cyclopropylmethoxy)-3,4-difluoro-benzamide (also known as CI-1040 or PD184352 and described in PCT Publication No. WO2000035436); N-[(2R)-2,3-Dihydroxypropoxy]-3,4-difluoro-2-[(2-fluoro-4-iodophenyl)amino]- benzamide (also known as PD0325901 and described in PCT Publication No. WO2002006213); 2,3-Bis[amino[(2-aminophenyl)thio]methylene]-butanedinitrile (also known as U0126 and described in US Patent No. 2,779,780); N-[3,4-Difluoro-2-[(2-fluoro-4-iodophenyl)amino]-6-methoxyphenyl]-1-[(2R)-2,3-dihydroxypropyl]- cyclopropanesulfonamide (also known as RDEA119 or BAY869766 and described in PCT Publication No. WO2007014011); (3S,4R,5Z,8S,9S,11E)-14-(Ethylamino)-8,9,16-trihydroxy-3,4-dimethyl-3,4,9, 19-tetrahydro-1H-2-benzoxacyclotetradecine-1,7(8H)-dione] (also known as E6201 and described in PCT Publication No. WO2003076424); 2'-Amino-3'-methoxyflavone (also known as PD98059 available from Biaffin GmbH & Co., KG, Germany); Vemurafenib (PLX-4032, CAS 918504-65-1); (R)-3-(2,3-Dihydroxypropyl)-6-fluoro-5-(2-fluoro-4-iodophenylamino)-8-methylpyrido[2,3-d]pyrimidine-4,7(3H,8H)-dione (TAK-733, CAS 1035555-63-5); Pimasertib (AS-703026, CAS 1204531-26-9); and Trametinib dimethyl sulfoxide (GSK-1120212, CAS 1204531-25-80).

[00270] Phosphoinositide 3-kinase (PI3K) inhibitors include but are not limited to, 4-[2-(1H-Indazol-4-yl)-6-[[4-(methylsulfonyl)piperazin-1-yl]methyl]thieno[3,2-d]pyrimidin-4-yl]morpholine (also known as GDC 0941 and described in PCT Publication Nos. WO 09/036082 and WO 09/055730); 2-Methyl-2-[4-[3-methyl-2-oxo-8-(quinolin-3-yl)-2,3-dihydroimidazo[4,5-c]quinolin-1-yl]phenyl]propionitrile (also known as BEZ 235 or NVP-BEZ 235, and described in PCT Publication No. WO 06/122806); 4-(trifluoromethyl)-5-(2,6-dimorpholinopyrimidin-4-yl)pyridin-2-amine (also known as BKM120 or NVP-BKM120, and described in PCT Publication No. WO2007/084786); Tozasertib (VX680 or MK-0457, CAS 639089-54-6); (5Z)-5-[[4-(4-Pyridinyl)-6-quinolinyl]methylene]-2,4-thiazolidinedione (GSK1059615, CAS 958852-01-2); (1E,4S,4aR,5R,6aS,9aR)-5-(Acetyloxy)-1-[(di-2-propenylamino)methylene]-4,4a,5,6,6a,8,9,9a-octahydro-11-hydroxy-4-(methoxymethyl)-4a,6a-dimethyl-cyclopenta[5,6]naphtho[1,2-c]pyran-2,7,10(1H)-trione (PX866, CAS 502632-66-8); and 8-Phenyl-2-(morpholin-4-yl)-chromen-4-one (LY294002, CAS 154447-36-6).

[00271] mTor include but are not limited to, Temsirolimus (Torisel®); Ridaforolimus (formally known as deferolimus, (1*R*,2*R*,4*S*)-4-[(2*R*)-2 [(1*R*,9*S*,12*S*,15*R*,16*E*,18*R*,19*R*,21*R*,23*S*,24*E*,26*E*,28*Z*,30*S*,32*S*,35*R*)-1,18-dihydroxy-19,30-dimethoxy-15,17,21,23, 29,35-hexamethyl-2,3,10,14,20-pentaoxo-11,36-dioxo-4-azatricyclo[30.3.1.0^{4,9}] hexatriaconta-16,24,26,28-tetraen-12-yl]propyl]-2-methoxycyclohexyl dimethylphosphinate, also known as AP23573 and MK8669, and described in PCT Publication No. WO 03/064383); Everolimus (Afinitor® or RAD001); Rapamycin (AY22989, Sirolimus®); Simapimod (CAS 164301-51-3); (5-{2,4-Bis[(3*S*)-3-methylmorpholin-4-yl]pyrido[2,3-*d*]pyrimidin-7-yl}-2-methoxyphenyl)methanol (AZD8055); 2-Amino-8-[*trans*-4-(2-hydroxyethoxy)cyclohexyl]-6-(6-methoxy-3-pyridinyl)-4-methyl-pyrido[2,3-*d*]pyrimidin-7(8*H*)-one (PF04691502, CAS 1013101-36-4); and *N*²-[1,4-dioxo-4-[[4-(4-oxo-8-phenyl-4*H*-1-benzopyran-2-yl)morpholinium-4-yl]methoxy]butyl]-L-arginylglycyl-L- α -aspartyl-L-serine-, inner salt (SF1126, CAS 936487-67-1).

[00272] In yet another aspect, the present invention provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate of the present invention in combination with one or more pro-apoptotics, including but not limited to, IAP inhibitors, Bcl2 inhibitors, MC11 inhibitors, Trail agents, Chk inhibitors.

[00273] For examples, IAP inhibitors include but are not limited to, LCL161, GDC-0917, AEG-35156, AT406, and TL32711. Other examples of IAP inhibitors include but are not limited to those disclosed in WO04/005284, WO 04/007529, WO05/097791, WO 05/069894, WO 05/069888, WO 05/094818, US2006/0014700, US2006/0025347, WO 06/069063, WO 06/010118, WO 06/017295, and WO08/134679, all of which are incorporated herein by reference.

[00274] BCL-2 inhibitors include but are not limited to, 4-[4-[[2-(4-Chlorophenyl)-5,5-dimethyl-1-cyclohexen-1-yl]methyl]-1-piperazinyl]-N-[[4-[[[(1*R*)-3-(4-morpholinyl)-1-[(phenylthio)methyl]propyl]amino]-3-[(trifluoromethyl)sulfonyl]phenyl]sulfonyl]benzamide (also known as ABT-263 and described in PCT Publication No. WO 09/155386); Tetrocarcin A; Antimycin; Gossypol ((-)-BL-193); Obatoclax; Ethyl-2-amino-6-cyclopentyl-4-(1-cyano-2-ethoxy-2-oxoethyl)-4Hchromone-3-carboxylate (HA14 – 1); Oblimersen (G3139, Genasense®); Bak BH3 peptide; (-)-Gossypol acetic acid (AT-101); 4-[4-[(4'-Chloro[1,1'-biphenyl]-2-yl)methyl]-1-piperazinyl]-N-[[4-[[[(1*R*)-3-(dimethylamino)-1-[(phenylthio)methyl]propyl]amino]-3-nitrophenyl]sulfonyl]-benzamide (ABT-737, CAS 852808-04-9); and Navitoclax (ABT-263, CAS 923564-51-6).

[00275] Proapoptotic receptor agonists (PARAs) including DR4 (TRAILR1) and DR5 (TRAILR2), including but are not limited to, Dulanermin (AMG-951, RhApo2L/TRAIL); Mapatumumab (HRS-ETR1, CAS 658052-09-6); Lexatumumab (HGS-ETR2, CAS 845816-02-6); Apomab (Apomab®); Conatumumab (AMG655, CAS 896731-82-1); and Tigatuzumab (CS1008, CAS 946415-34-5, available from Daiichi Sankyo).

[00276] Checkpoint Kinase (CHK) inhibitors include but are not limited to, 7-Hydroxystaurosporine (UCN-01); 6-Bromo-3-(1-methyl-1*H*-pyrazol-4-yl)-5-(3*R*)-3-piperidinyl-pyrazolo[1,5-*a*]pyrimidin-7-amine (SCH900776, CAS 891494-63-6); 5-(3-Fluorophenyl)-3-ureidothiophene-2-carboxylic acid N-[(*S*)-piperidin-3-yl]amide (AZD7762, CAS 860352-01-8); 4-[[[(3*S*)-1-Azabicyclo[2.2.2]oct-3-yl]amino]-3-(1*H*-benzimidazol-2-yl)-6-chloroquinolin-2(1*H*)-one (CHIR 124, CAS 405168-58-3); 7-Aminodactinomycin (7-AAD), Isogranulatimide, debromohymenialdisine; N-[5-Bromo-4-methyl-2-[(2*S*)-2-morpholinylmethoxy]-phenyl]-N'-(5-methyl-2-pyrazinyl)urea (LY2603618, CAS 911222-45-2); Sulforaphane (CAS 4478-93-7, 4-Methylsulfinylbutyl isothiocyanate); 9,10,11,12-Tetrahydro- 9,12-epoxy-1*H*-diindolo[1,2,3-*fg*:3',2',1'-*kl*]pyrrolo[3,4-*i*][1,6]benzodiazocine-1,3(2*H*)-dione (SB-218078, CAS 135897-06-2); and TAT-S216A (YGRKKRRQRRRLYRSPAMPENL), and CBP501 ((d-Bpa)sws(d-Phe-F5)(d-Cha)rrrqrr).

[00277] In one aspect, the present invention provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate of the present invention in combination with one or more FGFR inhibitors. For example, FGFR inhibitors include but are not limited to, Brivanib alaninate (BMS-582664, (*S*)-((*R*)-1-(4-(4-Fluoro-2-methyl-1*H*-indol-5-yloxy)-5-methylpyrrolo[2,1-*f*][1,2,4]triazin-6-yloxy)propan-2-yl)2-aminopropanoate); Vargatef (BIBF1120, CAS 928326-83-4); Dovitinib dilactic acid (TKI258, CAS 852433-84-2); 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea (BGJ398, CAS 872511-34-7); Danusertib (PHA-739358); and N-[2-[[4-(Diethylamino)butyl]amino]-6-(3,5-dimethoxyphenyl)pyrido[2,3-*d*]pyrimidin-7-yl]-N'-(1,1-dimethylethyl)-urea (PD173074, CAS 219580-11-7). In a specific embodiment, the present invention provides a method of treating cancer by administering to a subject in need thereof an antibody drug conjugate of the present invention in combination another FGFR2 inhibitor, such as 3-(2,6-dichloro-3,5-dimethoxyphenyl)-1-(6((4-(4-ethylpiperazin-1-yl)phenyl)amino)pyrimidin-4-yl)-1-methylurea (also known as BGJ-398); or 4-amino-5-fluoro-3-(5-(4-methylpiperazin-1-yl)-

1*H*-benzo[*d*]imidazole-2-yl)quinolin-2(1*H*)-one (also known as dovitinib or TKI-258).
 AZD4547 (Gavine *et al.*, 2012, Cancer Research 72, 2045-56, N-[5-[2-(3,5-Dimethoxyphenyl)ethyl]-2*H*-pyrazol-3-yl]-4-(3*R*,5*S*)-diethylpiperazin-1-yl)benzamide),
 Ponatinib (AP24534; Gozgit *et al.*, 2012, Mol Cancer Ther., 11; 690-99; 3-[2-(imidazo[1,2-*b*]pyridazin-3-yl)ethynyl]-4-methyl-N-{4-[(4-methylpiperazin-1-yl)methyl]-3-(trifluoromethyl)phenyl}benzamide, CAS 943319-70-8)

Pharmaceutical Compositions

[00278] To prepare pharmaceutical or sterile compositions including immunoconjugates, the immunoconjugates of the invention are mixed with a pharmaceutically acceptable carrier or excipient. The compositions can additionally contain one or more other therapeutic agents that are suitable for treating or preventing cancer (breast cancer, colorectal cancer, lung cancer, multiple myeloma, ovarian cancer, liver cancer, gastric cancer, pancreatic cancer, acute myeloid leukemia, chronic myeloid leukemia, osteosarcoma, squamous cell carcinoma, peripheral nerve sheath tumors schwannoma, head and neck cancer, bladder cancer, esophageal cancer, Barretts esophageal cancer, glioblastoma, clear cell sarcoma of soft tissue, malignant mesothelioma, neurofibromatosis, renal cancer, melanoma, prostate cancer, benign prostatic hyperplasia (BPH), gynacomastica, rhabdomyosarcoma and endometriosis).

[00279] Formulations of therapeutic and diagnostic agents can be prepared by mixing with physiologically acceptable carriers, excipients, or stabilizers in the form of, *e.g.*, lyophilized powders, slurries, aqueous solutions, lotions, or suspensions (see, *e.g.*, Hardman *et al.*, Goodman and Gilman's The Pharmacological Basis of Therapeutics, McGraw-Hill, New York, N.Y., 2001; Gennaro, Remington: The Science and Practice of Pharmacy, Lippincott, Williams, and Wilkins, New York, N.Y., 2000; Avis, *et al.* (eds.), Pharmaceutical Dosage Forms: Parenteral Medications, Marcel Dekker, NY, 1993; Lieberman, *et al.* (eds.), Pharmaceutical Dosage Forms: Tablets, Marcel Dekker, NY, 1990; Lieberman, *et al.* (eds.) Pharmaceutical Dosage Forms: Disperse Systems, Marcel Dekker, NY, 1990; Weiner and Kotkoskie, Excipient Toxicity and Safety, Marcel Dekker, Inc., New York, N.Y., 2000).

[00280] In a specific embodiment, the clinical service form (CSF) of the antibody drug conjugates of the present invention is a lyophilisate in vial containing the ADC, sodium succinate, and polysorbate 20. The lyophilisate can be reconstitute with water for injection, the solution

comprises the ADC, sodium succinate, sucrose, and polysorbate 20 at a pH of about 5.0. For subsequent intravenous administration, the obtained solution will usually be further diluted into a carrier solution.

[00281] Selecting an administration regimen for a therapeutic depends on several factors, including the serum or tissue turnover rate of the entity, the level of symptoms, the immunogenicity of the entity, and the accessibility of the target cells in the biological matrix. In certain embodiments, an administration regimen maximizes the amount of therapeutic delivered to the patient consistent with an acceptable level of side effects. Accordingly, the amount of biologic delivered depends in part on the particular entity and the severity of the condition being treated. Guidance in selecting appropriate doses of antibodies, cytokines, and small molecules are available (see, *e.g.*, Wawrzynczak, *Antibody Therapy*, Bios Scientific Pub. Ltd, Oxfordshire, UK, 1996; Kresina (ed.), *Monoclonal Antibodies, Cytokines and Arthritis*, Marcel Dekker, New York, N.Y., 1991; Bach (ed.), *Monoclonal Antibodies and Peptide Therapy in Autoimmune Diseases*, Marcel Dekker, New York, N.Y., 1993; Baert *et al.*, *New Engl. J. Med.* 348:601-608, 2003; Milgrom *et al.*, *New Engl. J. Med.* 341:1966-1973, 1999; Slamon *et al.*, *New Engl. J. Med.* 344:783-792, 2001; Beniaminovitz *et al.*, *New Engl. J. Med.* 342:613-619, 2000; Ghosh *et al.*, *New Engl. J. Med.* 348:24-32, 2003; Lipsky *et al.*, *New Engl. J. Med.* 343:1594-1602, 2000).

[00282] Determination of the appropriate dose is made by the clinician, *e.g.*, using parameters or factors known or suspected in the art to affect treatment or predicted to affect treatment. Generally, the dose begins with an amount somewhat less than the optimum dose and it is increased by small increments thereafter until the desired or optimum effect is achieved relative to any negative side effects. Important diagnostic measures include those of symptoms of, *e.g.*, the inflammation or level of inflammatory cytokines produced.

[00283] Actual dosage levels of the active ingredients in the pharmaceutical compositions of the present invention 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 ester, salt or 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 known in the medical arts.

[00284] Compositions comprising antibodies or fragments thereof of the invention can be provided by continuous infusion, or by doses at intervals of, *e.g.*, one day, one week, or 1-7 times per week, once every other week, once every three weeks, once every four weeks, once every five weeks, once every six weeks, once every seven weeks, or once every eight weeks. Doses may be provided intravenously, subcutaneously, topically, orally, nasally, rectally, intramuscular, intracerebrally, or by inhalation. A specific dose protocol is one involving the maximal dose or dose frequency that avoids significant undesirable side effects.

[00285] For the immunoconjugates of the invention, the dosage administered to a patient may be 0.0001 mg/kg to 100 mg/kg of the patient's body weight. The dosage may be between 0.0001 mg/kg and 20 mg/kg, 0.0001 mg/kg and 10 mg/kg, 0.0001 mg/kg and 5 mg/kg, 0.0001 and 2 mg/kg, 0.0001 and 1 mg/kg, 0.0001 mg/kg and 0.75 mg/kg, 0.0001 mg/kg and 0.5 mg/kg, 0.0001 mg/kg to 0.25 mg/kg, 0.0001 to 0.15 mg/kg, 0.0001 to 0.10 mg/kg, 0.001 to 0.5 mg/kg, 0.01 to 0.25 mg/kg or 0.01 to 0.10 mg/kg of the patient's body weight. The dosage of the antibodies or fragments thereof of the invention may be calculated using the patient's weight in kilograms (kg) multiplied by the dose to be administered in mg/kg.

[00286] Doses of the immunoconjugates the invention may be repeated and the administrations may be separated by at least 1 day, 2 days, 3 days, 5 days, 10 days, 15 days, 30 days, 45 days, 2 months, 75 days, 3 months, or at least 6 months. In a specific embodiment, doses of the immunoconjugates of the invention are repeated every 3 weeks.

[00287] An effective amount for a particular patient may vary depending on factors such as the condition being treated, the overall health of the patient, the method, route and dose of administration and the severity of side effects (see, *e.g.*, Maynard *et al.*, A Handbook of SOPs for Good Clinical Practice, Interpharm Press, Boca Raton, Fla., 1996; Dent, Good Laboratory and Good Clinical Practice, Urch Publ., London, UK, 2001).

[00288] The route of administration may be by, *e.g.*, topical or cutaneous application, injection or infusion by intravenous, intraperitoneal, intracerebral, intramuscular, intraocular, intraarterial, intracerebrospinal, intralesional, or by sustained release systems or an implant (see,

e.g., Sidman *et al.*, Biopolymers 22:547-556, 1983; Langer *et al.*, J. Biomed. Mater. Res. 15:167-277, 1981; Langer, Chem. Tech. 12:98-105, 1982; Epstein *et al.*, Proc. Natl. Acad. Sci. USA 82:3688-3692, 1985; Hwang *et al.*, Proc. Natl. Acad. Sci. USA 77:4030-4034, 1980; U.S. Pat. Nos. 6,350,466 and 6,316,024). Where necessary, the composition may also include a solubilizing agent or a local anesthetic such as lidocaine to ease pain at the site of the injection, or both. In addition, pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent. See, *e.g.*, U.S. Pat. Nos. 6,019,968, 5,985,320, 5,985,309, 5,934,272, 5,874,064, 5,855,913, 5,290,540, and 4,880,078; and PCT Publication Nos. WO 92/19244, WO 97/32572, WO 97/44013, WO 98/31346, and WO 99/66903, each of which is incorporated herein by reference their entirety.

[00289] A composition of the present invention may also be administered via one or more routes of administration using one or more of a variety of methods known in the art. As will be appreciated by the skilled artisan, the route and/or mode of administration will vary depending upon the desired results. Selected routes of administration for the immunoconjugates of the invention include intravenous, intramuscular, intradermal, intraperitoneal, subcutaneous, spinal or other parenteral routes of administration, for example by injection or infusion. Parenteral administration may represent modes of administration other than enteral and topical administration, usually by injection, and includes, without limitation, intravenous, intramuscular, intraarterial, intrathecal, intracapsular, intraorbital, intracardiac, intradermal, intraperitoneal, transtracheal, subcutaneous, subcuticular, intraarticular, subcapsular, subarachnoid, intraspinal, epidural and intrasternal injection and infusion. Alternatively, a composition of the invention can be administered via a non-parenteral route, such as a topical, epidermal or mucosal route of administration, for example, intranasally, orally, vaginally, rectally, sublingually or topically. In one embodiment, the immunoconjugates of the invention is administered by infusion. In another embodiment, the immunoconjugates of the invention is administered subcutaneously.

[00290] If the immunoconjugates of the invention are administered in a controlled release or sustained release system, a pump may be used to achieve controlled or sustained release (see Langer, *supra*; Sefton, CRC Crit. Ref Biomed. Eng. 14:20, 1987; Buchwald *et al.*, Surgery 88:507, 1980; Saudek *et al.*, N. Engl. J. Med. 321:574, 1989). Polymeric materials can be used to achieve controlled or sustained release of the therapies of the invention (see *e.g.*, Medical Applications of Controlled Release, Langer and Wise (eds.), CRC Pres., Boca Raton, Fla., 1974;

Controlled Drug Bioavailability, Drug Product Design and Performance, Smolen and Ball (eds.), Wiley, New York, 1984; Ranger and Peppas, J. Macromol. Sci. Rev. Macromol. Chem. 23:61, 1983; see also Levy *et al.*, Science 228:190, 1985; During *et al.*, Ann. Neurol. 25:351, 1989; Howard *et al.*, J. Neurosurg. 71:105, 1989; U.S. Pat. No. 5,679,377; U.S. Pat. No. 5,916,597; U.S. Pat. No. 5,912,015; U.S. Pat. No. 5,989,463; U.S. Pat. No. 5,128,326; PCT Publication No. WO 99/15154; and PCT Publication No. WO 99/20253. Examples of polymers used in sustained release formulations include, but are not limited to, poly(2-hydroxy ethyl methacrylate), poly(methyl methacrylate), poly(acrylic acid), poly(ethylene-co-vinyl acetate), poly(methacrylic acid), polyglycolides (PLG), polyanhydrides, poly(N-vinyl pyrrolidone), poly(vinyl alcohol), polyacrylamide, poly(ethylene glycol), polylactides (PLA), poly(lactide-co-glycolides) (PLGA), and polyorthoesters. In one embodiment, the polymer used in a sustained release formulation is inert, free of leachable impurities, stable on storage, sterile, and biodegradable. A controlled or sustained release system can be placed in proximity of the prophylactic or therapeutic target, thus requiring only a fraction of the systemic dose (see, *e.g.*, Goodson, in Medical Applications of Controlled Release, *supra*, vol. 2, pp. 115-138, 1984).

[00291] Controlled release systems are discussed in the review by Langer, Science 249:1527-1533, 1990). Any technique known to one of skill in the art can be used to produce sustained release formulations comprising one or more immunoconjugates of the invention. See, *e.g.*, U.S. Pat. No. 4,526,938, PCT publication WO 91/05548, PCT publication WO 96/20698, Ning *et al.*, Radiotherapy & Oncology 39:179-189, 1996; Song *et al.*, PDA Journal of Pharmaceutical Science & Technology 50:372-397, 1995; Cleek *et al.*, Pro. Int'l. Symp. Control. Rel. Bioact. Mater. 24:853-854, 1997; and Lam *et al.*, Proc. Int'l. Symp. Control Rel. Bioact. Mater. 24:759-760, 1997, each of which is incorporated herein by reference in their entirety.

[00292] If the immunoconjugates of the invention are administered topically, they can be formulated in the form of an ointment, cream, transdermal patch, lotion, gel, spray, aerosol, solution, emulsion, or other form well-known to one of skill in the art. See, *e.g.*, Remington's Pharmaceutical Sciences and Introduction to Pharmaceutical Dosage Forms, 19th ed., Mack Pub. Co., Easton, Pa. (1995). For non-sprayable topical dosage forms, viscous to semi-solid or solid forms comprising a carrier or one or more excipients compatible with topical application and having a dynamic viscosity, in some instances, greater than water are typically employed. Suitable formulations include, without limitation, solutions, suspensions, emulsions, creams,

ointments, powders, liniments, salves, and the like, which are, if desired, sterilized or mixed with auxiliary agents (*e.g.*, preservatives, stabilizers, wetting agents, buffers, or salts) for influencing various properties, such as, for example, osmotic pressure. Other suitable topical dosage forms include sprayable aerosol preparations wherein the active ingredient, in some instances, in combination with a solid or liquid inert carrier, is packaged in a mixture with a pressurized volatile (*e.g.*, a gaseous propellant, such as freon) or in a squeeze bottle. Moisturizers or humectants can also be added to pharmaceutical compositions and dosage forms if desired. Examples of such additional ingredients are well-known in the art.

[00293] If the compositions comprising the immunoconjugates are administered intranasally, it can be formulated in an aerosol form, spray, mist or in the form of drops. In particular, prophylactic or therapeutic agents for use according to the present invention can be conveniently delivered in the form of an aerosol spray presentation from pressurized packs or a nebuliser, with the use of a suitable propellant (*e.g.*, dichlorodifluoromethane, trichlorofluoromethane, dichlorotetrafluoroethane, carbon dioxide or other suitable gas). In the case of a pressurized aerosol the dosage unit may be determined by providing a valve to deliver a metered amount. Capsules and cartridges (composed of, *e.g.*, gelatin) for use in an inhaler or insufflator may be formulated containing a powder mix of the compound and a suitable powder base such as lactose or starch.

[00294] Methods for co-administration or treatment with a second therapeutic agent, *e.g.*, a cytokine, steroid, chemotherapeutic agent, antibiotic, or radiation, are known in the art (see, *e.g.*, Hardman *et al.*, (eds.) (2001) Goodman and Gilman's The Pharmacological Basis of Therapeutics, 10^{sup}.th ed., McGraw-Hill, New York, N.Y.; Poole and Peterson (eds.) (2001) Pharmacotherapeutics for Advanced Practice: A Practical Approach, Lippincott, Williams & Wilkins, Phila., Pa.; Chabner and Longo (eds.) (2001) Cancer Chemotherapy and Biotherapy, Lippincott, Williams & Wilkins, Phila., Pa.). An effective amount of therapeutic may decrease the symptoms by at least 10%; by at least 20%; at least about 30%; at least 40%, or at least 50%.

[00295] Additional therapies (*e.g.*, prophylactic or therapeutic agents), which can be administered in combination with the immunoconjugates of the invention may be administered less than 5 minutes apart, less than 30 minutes apart, 1 hour apart, at about 1 hour apart, at about 1 to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours

apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, at about 12 hours to 18 hours apart, 18 hours to 24 hours apart, 24 hours to 36 hours apart, 36 hours to 48 hours apart, 48 hours to 52 hours apart, 52 hours to 60 hours apart, 60 hours to 72 hours apart, 72 hours to 84 hours apart, 84 hours to 96 hours apart, or 96 hours to 120 hours apart from the immunoconjugates of the invention. The two or more therapies may be administered within one same patient visit.

[00296] In certain embodiments, the immunoconjugates of the invention can be formulated to ensure proper distribution in vivo. For example, the blood-brain barrier (BBB) excludes many highly hydrophilic compounds. To ensure that the therapeutic compounds of the invention cross the BBB (if desired), they can be formulated, for example, in liposomes. For methods of manufacturing liposomes, see, *e.g.*, U.S. Pat. Nos. 4,522,811; 5,374,548; and 5,399,331. The liposomes may comprise one or more moieties which are selectively transported into specific cells or organs, thus enhance targeted drug delivery (see, *e.g.*, Ranade, (1989) J. Clin. Pharmacol. 29:685). Exemplary targeting moieties include folate or biotin (see, *e.g.*, U.S. Pat. No. 5,416,016 to Low *et al.*); mannosides (Umezawa *et al.*, (1988) Biochem. Biophys. Res. Commun. 153:1038); antibodies (Bloeman *et al.*, (1995) FEBS Lett. 357:140; Owais *et al.*, (1995) Antimicrob. Agents Chemother. 39:180); surfactant protein A receptor (Briscoe *et al.*, (1995) Am. J. Physiol. 1233:134); p 120 (Schreier *et al.*, (1994) J. Biol. Chem. 269:9090); see also K. Keinanen; M. L. Laukkanen (1994) FEBS Lett. 346:123; J. J. Killion; I. J. Fidler (1994) Immunomethods 4:273.

[00297] The invention provides protocols for the administration of pharmaceutical composition comprising immunoconjugates of the invention alone or in combination with other therapies to a subject in need thereof. The therapies (*e.g.*, prophylactic or therapeutic agents) of the combination therapies of the present invention can be administered concomitantly or sequentially to a subject. The therapy (*e.g.*, prophylactic or therapeutic agents) of the combination therapies of the present invention can also be cyclically administered. Cycling therapy involves the administration of a first therapy (*e.g.*, a first prophylactic or therapeutic agent) for a period of time, followed by the administration of a second therapy (*e.g.*, a second prophylactic or therapeutic agent) for a period of time and repeating this sequential administration, i.e., the cycle,

in order to reduce the development of resistance to one of the therapies (*e.g.*, agents) to avoid or reduce the side effects of one of the therapies (*e.g.*, agents), and/or to improve, the efficacy of the therapies.

[00298] The therapies (*e.g.*, prophylactic or therapeutic agents) of the combination therapies of the invention can be administered to a subject concurrently.

[00299] The term "concurrently" is not limited to the administration of therapies (*e.g.*, prophylactic or therapeutic agents) at exactly the same time, but rather it is meant that a pharmaceutical composition comprising antibodies or fragments thereof the invention are administered to a subject in a sequence and within a time interval such that the antibodies of the invention can act together with the other therapy(ies) to provide an increased benefit than if they were administered otherwise. For example, each therapy may be administered to a subject at the same time or sequentially in any order at different points in time; however, if not administered at the same time, they should be administered sufficiently close in time so as to provide the desired therapeutic or prophylactic effect. Each therapy can be administered to a subject separately, in any appropriate form and by any suitable route. In various embodiments, the therapies (*e.g.*, prophylactic or therapeutic agents) are administered to a subject less than 15 minutes, less than 30 minutes, less than 1 hour apart, at about 1 hour apart, at about 1 hour to about 2 hours apart, at about 2 hours to about 3 hours apart, at about 3 hours to about 4 hours apart, at about 4 hours to about 5 hours apart, at about 5 hours to about 6 hours apart, at about 6 hours to about 7 hours apart, at about 7 hours to about 8 hours apart, at about 8 hours to about 9 hours apart, at about 9 hours to about 10 hours apart, at about 10 hours to about 11 hours apart, at about 11 hours to about 12 hours apart, 24 hours apart, 48 hours apart, 72 hours apart, or 1 week apart. In other embodiments, two or more therapies (*e.g.*, prophylactic or therapeutic agents) are administered to a within the same patient visit.

[00300] The prophylactic or therapeutic agents of the combination therapies can be administered to a subject in the same pharmaceutical composition. Alternatively, the prophylactic or therapeutic agents of the combination therapies can be administered concurrently to a subject in separate pharmaceutical compositions. The prophylactic or therapeutic agents may be administered to a subject by the same or different routes of administration.

EXAMPLES

[00301] The following examples and intermediates serve to illustrate the invention without limiting the scope thereof.

Example 1: Screening for anti-FGFR2/4 antibodies***Cell Lines***

[00302] Ba/F3 cells were purchased from DSMZ, KatoIII, SNU16, SNU5 and HCI-H716 cells were purchased from the American Type Culture Collection (ATCC) and NUGC3 cells were obtained from the Japanese Collection of Research Bioresources (JCRB) Cell Bank. All cell lines were routinely cultured in appropriate growth medium supplemented with fetal bovine serum (FBS) as recommended by their respective suppliers.

Generation of Recombinant Human, Cyno, Mouse and Rat FGFR Vectors

[00303] Human, mouse and rat FGFR extracellular domains were gene synthesized based on amino acid sequences from the GenBank or Uniprot databases (see Table 2). Cynomolgus FGFR2 and 4 ECD cDNA templates were gene synthesized based on amino acid sequences information generated using mRNA from various cyno tissues (*e.g.* Zyagen Laboratories; Table 3). All synthesized DNA fragments were cloned into appropriate expression vectors *e.g.* CMV based vectors or pCDNATM3.1. with C-terminal tags to allow for purification.

Table 2. Generation of FGFR expression vectors. Amino acid numbering is based on the accession numbers or seq IDs provided in the table

Name	Description	Accession Number	SEQ ID NO
Human FGFR1 IIIb D1-3	Human FGFR1 isoform IIIb, residues 22-373-TAG	NM 015850	121
Human FGFR2 IIIb D1-3	Human FGFR2 isoform IIIb, residues 22-378-TAG	NM_022970/ P21802-3	122
Human FGFR3 IIIb D1-3	Human FGFR3 isoform IIIb, residues 23-377-TAG	NM_000142	123
Human FGFR4 ECD	Human FGFR4 extracellular domain, residues 22-369-TAG	NM_002011; P22455	124
Human FGFR2 IIIc D1-3	Human FGFR2 isoform IIIc, residues 22-378-TAG	NM_022970/ P21802-1	125
Cynomolgus	Cynomolgus monkey FGFR2	Not applicable.	126

monkey FGFR2 IIIb D1-3	isoform IIIb, residues 22-378-TAG		
Mouse FGFR2 IIIb D1-3	Mouse FGFR2 isoform IIIb, residues 22-378-TAG	NM_010207	127
Rat FGFR2 IIIb D1-3	Rat FGFR2 isoform IIIb, residues 41-397-TAG	XM_001079450	128
Rat FGFR4	Rat FGFR4, residues 17-367-TAG	NM_001109904	129
Cynomolgus monkey FGFR4	Cynomolgus monkey FGFR4 , residues 17-366-TAG	Not applicable.	130
Mouse FGFR4	Mouse FGFR4, residues 17-366- TAG	Mouse NM_008011	131

Table 3: Sequences of cynomolgus FGFR2 and FGFR4 proteins

Construct	Amino acid sequence in one letter code	SEQ ID NO
Cynomolgus monkey FGFR2 IIIb D1-3	METDTLLLWVLLLWVPGSTGRPSFSLVEDTTLEPEEPPTKYQIS QPEVYVAAPGESLEVRCLLKDAAVISWTKDGVHLGPNNRTVLI GEYLQIKGATPRDSGLYACTATRTVDSETWYFMVNVTDAISSG DDEDDTDGAEDFVSENGNNKRAPYWTNTEKMEKRLHAVPAA NTVKFRCPAGGNPTPTMRWLKNGKEFKQEHRRIGGYKVRNQHW SLIMESVVP SDKGN YTCVVENEYGSINHTYHLDVVERSHPRPIL QAGLPANASTVVGGDVEFVCKVYSDAQPHIQWIKHVEKNGSK YGPDGLPYLKVLKHSGINSSNAEVLALFNVTEADAGEYICKVSN YIGQANQSAWLTVLPKQQAPGREKEITASPDYLEKLEFRHDSGL NDIFEAQKIEWHE	126
Cynomolgus monkey FGFR4	METDTLLLWVLLLWVPGSTGLEASEEVELEPCLAPSMEQQEQE LTVALGQPVRLCCGRAERGHWYKEGSRLAPAGRVRGWRGRL EIASFLPEDAGRYLCLARASMIVLQNVTLTIDDSLTSSNDDDPQ SHRDSSNGHIYPQQAPYWTHPQRMEKKLHAVPAGNTVKFRCP AAGNPTPTIRWLKDGQAFHGENRIGGIRLRHQHWSLVMESVVP SDRGTYTCLVENAVGSIRYNYLLDV LERSPHRPILQAGLPANTT AVVGSDVELLCKVYSDAQPHIQWLKHIVINGSSFGADGFPYVQ VLKTADINSSEVEVL YLRNVSAEDAGEYTCLAGNSIGLSYQSA WLTVLPEEDLTWTAATPEARYTDKLEFRHDSGLNDIFEAQKIE WHE	130

Expression of Recombinant FGFR Proteins

[00304] The desired FGFR recombinant proteins were expressed in HEK293 derived cell lines (293T) previously adapted to suspension culture and grown in a mix of serum-free medium, 50% HyClone™ SFM4Transfx-293 without L-glutamine (HyClone™) and 50% FreeStyle-293 (Gibco®). Both small scale and large scale protein production were via transient transfection and was performed in multiple shaker flasks (Nalgene®), up to 1 L each, with Polyethylenimine (PEI, 25K, AlfaAesar®) as a plasmid carrier. When biotinylated proteins were to be expressed via the C-terminal Avi tag, a plasmid carrying the gene for BirA enzyme were included at the ratio of 1:2 to the ECD protein plasmid. Total DNA and Polyethylenimine was used at a ratio of 1:3 (w:w). DNA to culture ratio was 1 mg/L. The cell culture supernatants were harvested 6 days post transfection, centrifuged and sterile filtered prior to purification.

Tagged Protein Purification

[00305] Recombinant tagged FGFR2 and FGFR4 ECD proteins (*e.g.*, APP-FGFR2 and APP-FGFR4 ECD) were purified by collecting the cell culture supernatant. An anti-APP column was prepared by coupling an anti-APP monoclonal antibody to CNBr activated Sepharose®4B at a final ratio of 10 mg antibody per mL of resin. Expression supernatant was applied to an anti-APP column at a flow rate of 1- 2 mL/ minute or by gravity flow. After base-line washing with PBS, bound material was eluted with 100 mM glycine (pH 2.7) and immediately dialyzed against PBS overnight and then sterile filtered. Protein concentrations were determined by measuring the absorbance at 280 nm and converting using the protein extinction coefficient calculated based on individual protein's amino acid sequences. The purified protein was then characterized by SDS-PAGE, analytical size exclusion chromatography (HPLC-SEC). For those that had more than >10% aggregates in the affinity purified preparation, a second step SEC purification were performed followed by confirmation characterizations.

Generation of Ba/F3 FGFR2 Cell Lines

[00306] To generate these cells, human FGFR2-IIIb-C3 (NM_022970) was cloned into a pENTR™ TOPOD® vector (Invitrogen catalog #K2400-20) and then into a pLenti6 DEST™ vector (Invitrogen catalog #V49610) under a CMV promoter. Virus was then packaged and Ba/F3 cells (DSMZ catalog #ACC300) were spin infected with the lentivirus, followed by treatment with 20 µg blasticidin (*e.g.* Invitrogen, catalog # A11139-03 or Cellgro®, catalog # 30-100-RB) and selection with IL3 (R&D systems catalog #403-ml-010) for 5 days in a tissue

culture incubator at 37 °C and 5% CO₂. Surviving cells were then grown in medium (RPMI (Invitrogen catalog #11875-093) supplemented with 10% FBS (Clontech catalog #631101, 2 µg/ml heparin (Sigma, catalog # H3149) and 20 µg/ml blasticidin (*e.g.* Invitrogen, catalog # A11139-03 or Cellgro®, catalog # 30-100-RB)) but without IL3 for 1 month. Surviving cells were then dilution cloned to generate clonal cell populations which were then used for subsequent studies.

HuCAL PLATINUM[®] Pannings

[00307] For the selection of antibodies recognizing human FGFR2, multiple panning strategies were utilized. Therapeutic antibodies against human FGFR proteins were generated by the selection of clones that bound to FGFR2 using as a source of antibody variant proteins a commercially available phage display library, the Morphosys HuCAL PLATINUM[®] library. The phagemid library is based on the HuCAL[®] concept (Knappik *et al.*, 2000, J Mol Biol 296: 57-86) and employs the CysDisplay[™] technology for displaying the Fab on the phage surface (WO01/05950).

[00308] For the isolation of anti-FGFR2 antibodies, several different panning strategies were employed, using solid phase, solution, whole cell and differential whole cell approaches.

Solid Phase Panning on Recombinant FGFR2

[00309] Prior to the antigen selection process a coating check ELISA was performed to determine the optimal coating concentration for the antigen. Different recombinant FGFR2 proteins with various tags were used in the solid phase panning approach by coating on Maxisorp[™] plates (Nunc®) either via passive adsorption or via capture antibodies targeting the respective tag of the antigen. Alternatively Reacti-Bind[™] NeutrAvidin[™]-coated Polystyrene Strip Plates (Pierce) were used to capture biotinylated FGFR2 antigen. An appropriate number (dependent on the number of sub-library pools) of wells of a 96-well Maxisorp[™] plate (Nunc®) were coated with antigen overnight at 4°C. The coated wells were blocked with PBS (phosphate buffered saline)/5% milk powder. For each panning, about HuCAL PLATINUM[®] phage-antibodies were blocked. After the blocking procedure, pre-blocked phage mix was added to each antigen coated and blocked well and incubated for 2 hours (h) at room temperature (RT) on a microtiter plate (MTP) shaker. Afterwards, unspecific bound phage was washed off by several washing steps. For elution of specifically bound phage, 25 mM DTT (Dithiothreitol) was added for 10 minutes (min) at RT. The DTT eluates were used for infection of *E. coli* (Escherichia coli)

TG-1 cells.. After infection, the bacteria were plated on LB (lysogeny broth)/Cam (chloramphenicol) agar plates and incubated overnight at 30°C. Colonies were scraped off the plates and were used for phage rescue, polyclonal amplification of selected clones, and phage production. Each FGFR2 solid phase panning strategy comprised individual rounds of panning and contained unique antigens, antigen concentrations, buffer compositions and washing stringencies.

Solution Panning on Recombinant FGFR2 with Streptavidin-Coupled Magnetic Beads

[00310] A prerequisite for solution panning approaches was the biotinylation of the antigen and confirmation of retained activity of biotinylated antigen. During solution panning, the Fab displaying phage and the biotinylated antigen were incubated in solution which facilitated the accessibility of the antigen by the phage

[00311] For each phage pool, Streptavidin beads (Dynabeads[®] M-280 Streptavidin; Invitrogen) were blocked in 1x Chemiblocker[™]. In parallel, for each panning, about HuCAL PLATINUM[®] phage-antibodies were blocked with an equal volume of 2x Chemiblocker[™]/ 0.1% Tween20. Then, a certain concentration of biotinylated antigen (*e.g.* 100 nM) was added to the pre-adsorbed and blocked phage particles and incubated for 1-2 h at RT on a rotator. The phage-antigen complexes were captured using blocked Streptavidin beads and phage particles bound to the Streptavidin beads were collected with a magnetic separator. Unspecific bound phages were washed off by several washing steps. For elution of specifically bound phage from Streptavidin beads, 25 mM DTT were added for 10 min at RT. The DTT eluate was processed as described for the solid phase pannings.

[00312] Each FGFR2 solution phase panning strategy comprised individual rounds of panning and contained unique antigens, antigen concentrations and washing stringencies.

Whole Cell Panning on FGFR2 Overexpressing Cells

[00313] For each cell panning, HuCAL PLATINUM[®] phage-antibodies were pre-blocked in PBS/FCS. In parallel, 0.5-1.0x10⁷ target cells per phage pool (Ba/F3 cells stably transfected with FGFR2, Kato-III, SNU16, H716) showing overexpression of FGFR2 were resuspended in PBS/FCS on ice.

[00314] The blocked target cells were spun down, re-suspended in the pre-blocked phage particles and incubated for 2 h at 4°C on a rotator. The phage-cell complexes were washed in PBS/FCS. Elution of specifically bound phage from target cells was performed by acidic elution

with glycine buffer, pH 2.2. After centrifugation, the supernatant (eluate) was neutralized by adding unbuffered Tris. The final phage containing supernatant was used for infection of *E. coli* TG1 culture. The following steps were done as described under the section solid phase pannings.

[00315] In more detail, either whole cell pannings were performed, where each panning round was performed with cells or, alternatively, differential whole cell pannings were performed, meaning either cells or recombinant protein was used in consecutive panning rounds. The selection rounds on recombinant antigen were performed as described for solid phase or solution pannings.

Maturation Pannings

[00316] In order to obtain FGFR2 specific antibodies with increased affinities, maturation pannings were performed (Prassler *et al.*, 2009, Immunotherapy, 1: 571-583). For this purpose, standard pannings (solid phase and solution phase panning) were performed with different FGFR2 antigens as described above.

[00317] After the panning, Fab-encoding fragments of phage derived pMORPH30[®] vector DNA were digested with distinct specific restriction enzymes to generate either LCDR3 or HCDR2 matured libraries. The insertes were replaced using TRIM[™] technology (Virnekas *et al.*, 1994, Nucleic Acids Research 22: 5600-5607).

[00318] The generated libraries were amplified and subjected to two more rounds of panning with increased washing stringency and reduced antigen concentrations.

Subcloning and Microexpression of Selected Fab Fragments

[00319] To facilitate rapid expression of soluble Fab, the Fab encoding inserts of the selected HuCAL PLATINUM[®] phage were subcloned from pMORPH[®]30 display vector into pMORPH[®]x11 expression vector pMORPH[®]x11_FH.

[00320] After transformation of *E. coli* TG1-F⁻ single clone expression and preparation of periplasmic extracts containing HuCAL[®]-Fab fragments were performed as described previously (Rauchenberger *et al.*, 2003 J Biol Chem 278: 38194-38205).

ELISA Screening

[00321] Using ELISA screening, single Fab clones are identified from panning output for binding to the target antigen. Fabs are tested using Fab containing crude *E. coli* lysates.

[00322] Maxisorp[™] (Nunc[®]) 384 well plates were coated with FGFR antigens of interest (either via passive adsoption or via capure antibodies targeting the respective tag of the antigen) in

PBS at their previous determined saturation concentration. Alternatively, Reacti-Bind™ NeutrAvidin™-coated Polystyrene Strip Plates (Pierce) were used to capture biotinylated FGFR2 antigen.

[00323] After blocking of plates with 5% skim milk powder in PBS, Fab-containing *E. coli* lysates were added. Binding of Fabs was detected by F(ab)₂ specific goat anti-human IgG conjugated to alkaline phosphatase (diluted 1:5000) using Attophos® fluorescence substrate (Roche, catalog #11681982001). Fluorescence emission at 535 nm was recorded with excitation at 430 nm.

FACS Screening (Fluorescence Activated Cell Sorting)

[00324] In FACS screening, single Fab clones binding to cell surface expressed antigen are identified from the panning output. Fabs are tested for cell binding using Fab containing crude *E. coli* lysates.

[00325] In these studies, 100 µl of cell-suspension was transferred into a fresh 96-well plate (resulting in 1x10⁵ cells/well). Target cell suspension containing plates were centrifuged and the supernatant was discarded. The remaining cell pellets were re-suspended and 50 µl of Fab containing bacterial extracts was added to the corresponding wells.

[00326] Alternatively, the cell pellet was re-suspended and 50 µl FACS buffer (PBS, 3% FCS) and same volume of Fab containing bacterial extracts was added to the corresponding wells.

[00327] The cell-antibody suspensions were then incubated on ice for 1 hour. Following incubation, cells were spun down and washed three times with 200 µl FACS buffer. After each washing step, cells were centrifuged and carefully re-suspended.

[00328] Secondary detection antibody (PE conjugated goat anti human IgG; Dianova) was added and samples were incubate on ice and subsequently washed according to Fab incubation. Fluorescence intensity was determined in a FACSArray™ instrument.

Expression and Purification of HuCAL® Fab Fragments

[00329] Expression of Fab fragments was performed in *E. coli* TG1 F- cells. Cultures were shaken at 30°C for 18 h. Cells were harvested and disrupted using a combination of lysozyme and Bug Buster® Protein Extraction Reagent (Novagen, Germany). His6-tagged Fab fragments were isolated via IMAC (Qiagen®, Germany) and protein concentrations were determined by UV-spectrophotometry. The purity of representatively selected samples was

analyzed in denaturing, reducing 15% SDS-PAGE (sodium dodecyl sulfate polyacrylamide gel electrophoresis).

[00330] The homogeneity of Fab preparations was determined in native state by size exclusion chromatography (HP-SEC) with calibration standards.

Conversion to IgG and IgG Expression

[00331] In order to express full length IgG, variable domain fragments of heavy (VH) and light chains (VL) were subcloned from Fab expression vectors into appropriate pMorph®_hIg vectors for human IgG1. Alternatively, eukaryotic HKB11 cells were transfected with pMORPH®4 expression vector DNA.. The cell culture supernatant was harvested 3 or 7 days post transfection. After sterile filtration, the solution was subjected to Protein A affinity chromatography (MabSelect SURE™, GE Healthcare) using a liquid handling station. If not otherwise stated, buffer exchange was performed to 1x Dulbecco's PBS (pH 7.2, Invitrogen) and samples were sterile filtered (0.2 µm pore size). Protein concentrations were determined by UV-spectrophotometry and purity of IgGs was analyzed under denaturing, reducing conditions using a Caliper Labchip® System or in SDS-PAGE.

Bioassays

[00332] Anti-FGFR antibodies obtained following the panning processes described above were evaluated in the assays exemplified below:

BaF3/CMV-FGFR2IIIb-C3 Cell Proliferation Assay

[00333] To determine the capacity of anti-FGFR2 antibodies to inhibit FGF1-dependent cell proliferation, a proliferation assay using an engineered Ba/F3 cell line (BaF3/CMV-FGFR2IIIb-C3), which was stably transduced with a chimera of full-length FGFR2IIIb receptor ECD and FGFR1-intracellular domain harboring the respective kinase domains. Addition of human FGF1 promoted cell proliferation in this cell line.

[00334] Cells were re-suspended in full-growth medium (RPMI + 10% FCS + 30ng/ml FGF1 + 2µg/ml Heparin + 20µg/ml Blasticidin) and seeded into flat-bottomed white 96-well assay plates (Corning® Costar, #3903) at a cell density of 8×10^3 cells/well in 80 µl and incubated at 37°C and 5% CO₂ overnight.

[00335] The next day, the HuCAL® antibodies (Fab or IgG) were diluted in full-growth medium to the desired concentrations (5 fold concentrated). 20 µl of the antibody solutions were added to the seeded cells of the previous day and the cells were cultivated for 72 hr. After 72hr, 100 µl Cell Titer-Glo™ Luminescent Cell Viability Assay Reagent (#G7571; Promega) was

added to each well and incubated for 15 min at RT slightly shaking with subsequent read-out of the luminescence in a luminometer (GeniosPro, Tecan). For determination of the half maximal inhibitory concentration (IC₅₀ values), Fab/IgG titration was performed and IC₅₀ was calculated using GraphPad Prism.

[00336] For the evaluation of a potential agonistic activity of the HuCAL[®] IgGs, as determined by the ability of the antibodies to promote proliferation of the engineered BaF/3 cells in the absence of exogenous FGF1, the cells and IgGs were diluted in dilution medium (full-growth medium without FGF1). Seeding of the cells and the addition of the IgGs was done at the same day.

Affinity Determination

[00337] For K_D determinations, monomer fractions of antibody protein were used (at least 90% monomer content, analyzed by analytical SEC; Superdex[™]75 (Amersham Pharmacia) for Fab, or Tosoh G3000SWXL (Tosoh Bioscience) for IgG, respectively).

(a) Solution Equilibrium Titration (SET) Method for KD Determination Using Sector[®] Imager 6000 (Mesoscale discovery[®])

[00338] Affinity determination in solution was basically performed as described in the literature (Friguet *et al.*, 1985 J Immunol Methods 77: 305-319). In order to improve the sensitivity and accuracy of the SET method, it was transferred from classical ELISA to ECL based technology (Haenel *et al.*, 2005 Anal Biochem 339: 182-184). K_D determination of HuCAL[®]_IgG was performed as described using the following reagents: biotinylated hFGFR2. was coated at 0.5 µg/ml in PBS overnight at 4°C on standard MSD plates.

[00339] After washing the MSD plate and adding 30 µl/well MSD Read Buffer T with surfactant, electrochemiluminescence signals were detected using a Sector[®] Imager 6000 (MesoScale Discovery[®], Gaithersburg, MD, USA).

[00340] The data was evaluated with XLfit[™] (IDBS) software applying customized fitting models. For K_D determination of Fab molecules the following fit model was used (according to (Haenel *et al.*, 2005 Anal Biochem 339: 182-184):

$$y = B_{\max} - \left(\frac{B_{\max}}{2[Fab]_t} \left([Fab]_t + x + K_D - \sqrt{([Fab]_t + x + K_D)^2 - 4x[Fab]_t} \right) \right)$$

[Fab]_t: applied total Fab concentration

x: applied total soluble antigen concentration (binding sites)

B_{max}: maximal signal of Fab without antigen

K_D : affinity

[00341] For K_D determination of IgG molecules the following fit model for IgG was used (modified according to (Piehler *et al.*, 1997 J Immunol Methods 201: 189-206)) and a summary of the data is presented in FIG. 1:

$$y = \frac{2B_{\max}}{[IgG]} \left(\frac{[IgG]}{2} - \frac{\left(\frac{x + [IgG] + K_D}{2} - \sqrt{\frac{(x + [IgG] + K_D)^2}{4} - x[IgG]} \right)^2}{2[IgG]} \right)$$

[IgG]: applied total IgG concentration

x: applied total soluble antigen concentration (binding sites)

$B_{\max}$: maximal signal of IgG without antigen

K_D : affinity

Summary of Panning Strategies and Screening

[00342] In total, 28 different panning strategies were employed using recombinant antigen material as well as cell lines as outlined above. Using the assays described above, panning outputs were screened for specificity and cross-reactivity and 935 clones were selected for sequencing. Following the screening process described, 15 antibodies were selected for further characterization as described in the examples below. Of these antibodies, 12433, 12947, 10846 and 12931 were found to bind to the FGFR2 IIIb isoform (Uniprot Accession # P21802-3), 10164, 11725, 10220, 11723, and 12944 were found to bind to both human FGFR2 IIIb (Uniprot Accession # P21802-3) and IIIc isoforms (Uniprot Accession # P21802-1), and 12425, 12422, 12439, 10918, 10923 and 11722 were found to bind to both human FGFR2 IIIb (Uniprot Accession # P21802-3) and IIIc isoforms (Uniprot Accession # P21802-1) and also human FGFR4 (Uniprot Accession # P22455).

Example 2: Affinity determination

[00343] Affinity of the antibodies to FGFR2 species orthologues and also to FGFR4 was determined using Biacore® technology using a Biacore® T100 instrument (GE Systems) and with CM5 sensor chips.

[00344] Briefly, HBS-EP⁺ (0.01 M HEPES, pH 7.4, 0.15 M NaCl, 0.005% Surfactant P20) supplemented with 0.5 mg/ml of BSA and 10 µg/ml heparin was used as running buffer for all

experiments. The immobilization level and analyte interactions were measured by response unit (RU). Pilot experiments were performed to test and confirm the feasibility of the immobilization of anti-human Fc antibody and the capture of the test antibodies.

[00345] For kinetic measurements, experiments were performed in which the antibodies were immobilized to the sensor chip surface and the ability of the FGFR proteins described above listed in Table 1 to bind in free solution was determined. Briefly, 50 µg/ml of anti-human Fc antibody at pH 4.75 was immobilized on a CM5 sensor chip through amine coupling at flow rate of 10 µl/minute on all four flow cells to reach 6000 RUs. 3 µg/ml of test antibodies were then injected at 10 µl/min for 10 s. Immobilization levels of antibodies were generally kept below 250 RUs. Subsequently, 0.078 -100 nM of FGFR receptors, diluted in a 2-fold series, were injected at a flow rate of 80 µl/min for 7 min over both reference and test flow cells. Dissociation of the binding was followed for 10 min using HBS-EP⁺ supplemented with 0.5 mg/ml of BSA and 10 µg/ml heparin as running buffer. After each injection cycle, the chip surface was regenerated with 10 mM glycine, pH 2.0 at 60 µl/min for 70 s. All experiments were performed at 25 °C and the response data were globally fitted with a simple 1:1 interaction model (using evaluation software version 1.1 (GE Systems)) to obtain estimates of on rate (k_a), off-rate (k_d) and affinity (K_D).

[00346] A summary of the affinity estimates obtained against FGFR2 IIIb species orthologues and FGFR4 is presented in Table 4. It was found that all of the antibodies bound to the species orthologues of FGFR2 evaluated and that a subset of the antibodies also bound to human FGFR4. In addition, some of the antibodies (10846, 12433, 12931 and 12947) were unable to bind to the IIIc isoform of human FGFR2 (Uniprot Accession # P21802). All of the antibodies were specific to FGFR2 or bound to both FGFR2 and FGFR4 and none of the antibodies were found to bind appreciably to FGFR1 or FGFR3.

Table 4. Affinity estimates obtained against FGFR2 IIIb species orthologues and FGFR4

Antibody ID	Affinity estimate (K_D) (M)				
	Human FGFR2 IIIb	Mouse FGFR2 IIIb	Rat FGFR2 IIIb	Cyno FGFR2 IIIb	Human FGFR4
10164	1.9E-08	3.3E-08	3.5E-08	1.6E-08	N.B.
10220	1.6E-08	2.6E-08	2.6E-08	2.6E-08	N.B.
10846	1.4E-12	1.5E-08	1.3E-08	2.3E-08	N.B.
10918	7.7E-10	1.1E-09	1.3E-09	1.3E-09	1.8E-09
10923	3.7E-09	4.0E-09	5.5E-09	4.5E-09	6.4E-09

11722	1.3E-08	1.8E-08	2.1E-08	1.3E-08	1.1E-08
11723	1.8E-08	2.8E-08	5.3E-08	1.7E-08	N.B.
11725	2.6E-08	1.3E-07	1.4E-07	7.3E-09	N.B.
12422	5.1E-09	7.7E-09	9.7E-09	7.4E-09	9.5E-10
12425	1.4E-08	1.6E-08	2.2E-08	1.2E-08	3.9E-09
12433	5.6E-09	8.0E-09	1.2E-08	1.2E-08	N.B.
12439	1.5E-08	1.5E-08	1.6E-08	5.6E-09	4.3E-09
12931	3.4E-09	1.2E-09	2.9E-09	2.4E-09	N.B.
12944	9.4E-09	6.9E-09	1.1E-08	1.0E-08	N.B.
12947	2.7E-09	3.1E-09	5.1E-09	2.4E-09	N.B.

Example 3: Assessment of functional activity of anti-FGFR antibodies

[00347] The ability of the purified antibodies to act as either agonists or antagonists of FGFR2 was evaluated using a Baf cell system in which Baf cells were transduced to overexpress FGFR2.

[00348] To evaluate potential agonistic properties of the antibodies, Baf-FGFR2 cells were washed twice in PBS and re-suspended in dilution medium (RPMI supplemented with 10% FBS, 2 µg/ml heparin (Sigma, catalog # H3149) and 20 µg/ml blasticidin (*e.g.* Invitrogen, catalog # A11139-03 or Cellgro®, catalog # 30-100-RB) prior to seeding in 96 well plates (Costar catalog # 3904) at 8000 cells/well in 90 µl dilution medium. In each assay, one plate was designated a “day 0” plate: to each well of these plates, an additional 10 µl of dilution medium was added, followed 80 µl/well of Cell titer Glo® reagent (Promega #G7573). Assay plates were shaken gently for 10 min and the resulting luminescence intensity was measured using a Perkin Elmer EnVision® 2101 plate reader. Serial dilutions were prepared for each antibody as 10X solutions in dilution medium and 10 µl was added to the appropriate wells. In addition to the test antibodies, FGF1 (Peprotech, catalog # 100-17A; final assay concentration 0-30 nM) a commercially available anti-FGFR2 antibody (R&D systems, catalog # MAB6841) and a non-FGFR2 binding antibody were included as control reagents. Assay plates were incubated for 3 days at 37°C with 5% CO₂. Following this incubation, the plates were brought to room temperature for around 30 min and 80 µl/well of Cell titer Glo® reagent (Promega #G7573) was added. Plates were then shaken gently for 10 min and the resulting luminescence intensity was measured using a Perkin Elmer EnVision® 2101 plate reader. To determine the effect of the antibodies on cell proliferation, raw

luminescence values were averaged across replicates and compared to day 0 controls. 10846, 11725 and 12439 were not evaluated in these studies but none of the other antibodies tested were able to substitute for FGF1 and maintain cell growth. A summary of the data is presented in FIG. 2 (A)/(B).

[00349] The Baf cell system was also used to assess the potential of the antibodies to act as antagonists of FGFR2 receptor signaling. In these studies, Baf-FGFR2 cells were plated as described in the agonism studies above with the addition of 30 µg/ml FGF1 (Peprotech, catalog # 100-17A) to the dilution medium. Serial dilutions were prepared for each antibody as 4X solutions in dilution medium with 30 µg/ml FGF1 and 25 µl was added to the appropriate wells. In addition to the test antibodies, a commercially available anti-FGFR2 antibody (R&D systems, catalog # MAB6841) and a non-FGFR2 binding antibody were included as control reagents. Assay plates were incubated for 3 days at 37°C with 5% CO₂. Following this incubation, the plates were brought to room temperature for around 30 min and 80 µl/well of Cell titer Glo® reagent (Promega #G7573) was added. Plates were then shaken gently for 10 min and the resulting luminescence intensity was measured using a Perkin Elmer EnVision® 2101 plate reader. To determine the effect of the antibodies on relative cell proliferation, raw luminescence values were averaged across replicates and compared. 10846, 11725 and 12439 were not evaluated in these studies. The effects of the other antibodies tested are presented in FIG. 3(A)/(B) and demonstrate that of the clones evaluated 10918, 10923, 12931, 12944, 12947 and 12422 inhibited the proliferation of the engineered Baf cells by greater than 50% at concentrations of less than 100 nM.

Example 4: Preparation of ADCs

Preparation of the DM1 Conjugates by One-Step Process

[00350] Antibody 12425 was diafiltered into a reaction buffer (15 mM potassium phosphate, 2 mM EDTA, pH 7.6) via Tangential Flow Filtration (TFF#1) prior to the start of the conjugation reaction. Subsequently, antibody 12425 (5.0 mg/mL) was mixed with DM1 (5.6-fold molar excess relative to the amount of antibody) and then with SMCC (4.7 fold excess relative to the amount of antibody). The reaction was performed at 20° C in 15 mM potassium phosphate buffer (pH 7.6) containing 2 mM EDTA and 10% DMA for approximately 16 hours. The reaction was quenched by adding 1 M acetic acid to adjust the pH to 5.50. After pH adjustment, the

reaction mixture was filtered through a multi-layer (0.45/0.22 μm) PVDF filter and purified and diafiltered into a 20 mM succinate buffer (pH 5.0) containing 8.22% sucrose using Tangential Flow Filtration (TFF#2). The instrument parameters for the Tangential Flow Filtration are listed in Table 5 below.

Table 5. Instrument parameters for the Tangential Flow Filtration

TFF Parameter	TFF#1 Set Point	TFF#2 Set Point
Bulk Concentration (C_b – g/L)	20	20
TMP (psi)	12-18	12-18
Feed Flow rate (LMH)	324	324
Membrane Load (g/m ²)	110 – 150	110 – 150
Diavolumes	10	14
Diafiltration Buffer	15 mM potassium phosphate, 2 mM EDTA, pH 7.6	20 mM Succinate, 8.22% Sucrose, pH 5.0
Temperature (°C)	RT (20 – 25)	RT (20 – 25)

[00351] Conjugates obtained from the process described above was analyzed by: UV spectroscopy for cytotoxic agent loading (Maytansinoid to Antibody Ratio, MAR); SEC-HPLC for determination of conjugate monomer; and reverse-phase HPLC or hydrophobic shielded phase (Hisep)-HPLC for free maytansinoid percentage. The data is shown in Table 6.

Table 6. Properties of 12425-MCC-DM1

Sample	MAR	Monomer (%)	Total Free Maytansinoid (%)
12425-MCC-DM1	3.6	98.0	1.0

Preparation of DM1 Conjugates by in situ Process

[00352] The conjugates of the present invention can also be prepared by in situ process according to the following procedures. Antibodies (21 clones) were conjugated to DM1 using the sulfosuccinimidyl 4-(N-maleimidomethyl) cyclohexane-1-carboxylate (sulfo-SMCC) linker. Stock solutions of DM1 and sulfo-SMCC heterobifunctional linker were prepared in DMA. Sulfo-SMCC and DM1 thiol were mixed together to react for 10 minutes at 25 °C in DMA containing 40% v/v of aqueous 50 mM succinate buffer, 2 mM EDTA, pH 5.0, at the ratio of DM1 to linker of 1.3:1 mole equivalent and a final concentration of DM1 of 1.95 mM. The

antibody was then reacted with an aliquot of the reaction to give a mole equivalent ratio of SMCC to Ab of around 6.5:1 under final conjugation conditions of 2.5 mg/mL of Ab in 50 mM EPPS, pH 8.0 and 10% DMA (v/v). After approximately 18 hours at 25 °C, the conjugation reaction mixture was purified using a SEPHADEX™ G25 column equilibrated with 10 mM succinate, 250 mM glycine, 0.5% sucrose, 0.01% Tween 20, pH 5.5.

Table 7. Properties of DM1-conjugated antibodies

Clone name	Linker excess	MAR	Monomer (%)	Yield (%)	Free drug (%)
10164	6.8	3.5	98	88	<0.5
10220	7.5	3.55	98	96	0.5
10553	6.4	3.7	100	64	<0.5
10554	6.3	3.5	100	79	<0.5
10846	6.3	3.4	99	71	<0.5
10918	6.4	3.6	98	99	0.5
10923	6.2	3.5	100	95	0.7
10925	7.9	3.5	99	70	<0.5
11722	9.5	3.5	99	84	3.7
11723	7.3	3.3	99	64	<0.5
11725	8.2	3.4	99	74	<0.5
11729	9.5	3.2	93	30	0.7
12422	6.2	4.0	99	60	<0.5
12425	6.1	3.5	99	70	<0.5
12433	6.1	3.4	99	82	<0.5
12435	7.0	3.4	99	75	<0.5
12438	7.7	3.4	99	75	<0.5

12439	6.4	3.5	99	99	0.5
12931	6.4	3.8	99	80	0.5
12944	7.8	3.5	99	38	1.6
12947	6.7	3.8	99	99	<0.5

Preparation of ADCs with the SPDB linker

[00353] Antibodies 12422, 12425 and 12433 (8 mg/ml) was modified with N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB, 5.0, 5.5 and 4.9 fold molar excess respectively) for 120 minutes at 25 °C in 50 mM potassium phosphate buffer (pH 7.5) containing 50 mM NaCl, 2 mM EDTA, and 5% DMA. The modified Ab without purification was subsequently conjugated to DM4 (1.7 fold molar excess over the unbound linker) at a final modified antibody concentration of 4 mg/mL in 50 mM potassium phosphate buffer (pH 7.5) containing 50 mM NaCl, 2 mM EDTA, and 5% DMA for 18 hours at 25 °C. The conjugation reaction mixture was purified using a SEPHADEX™ G25 column equilibrated and eluted with 10 mM succinate, 250 mM glycine, 0.5% sucrose, 0.01% Tween 20, pH 5.5.

Table 8. Properties of DM4-conjugated antibodies

Clone name	Linker excess	MAR	Monomer (%)	Yield (%)	Free drug (%)
12422	5.0	3.6	99	85	0.5
12425	5.5	3.6	98	>90	<0.5
12433	4.9	4.0	99	75	0.5

Preparation of ADCs with the CX1-1 linker

[00354] Antibody 12425 (5.0 mg/mL) was mixed with DM1 (7.15-fold molar excess relative to the amount of antibody) and then with CX1-1 (5.5-fold excess relative to the amount of antibody). The reaction was performed at 25°C in 60 mM EPPS [4-(2-Hydroxyethyl)-1-piperazinepropanesulfonic acid] buffer (pH 8.5) containing 2 mM EDTA and 5% DMA for approximately 16 hours. The reaction mixture was then purified using a SEPHADEX™ G25

column equilibrated and eluted in 10 mM succinate, 250 mM glycine, 0.5% sucrose, 0.01% Tween 20, pH 5.5.

Table 9. Properties of CX1-1/DM1 conjugated 12425

Clone name	Linker excess	MAR	Monomer (%)	Yield (%)	Free drug (%)
12425	5.5 X	3.4	97	>90	0.1

Example 5: Affinity of ADCs relative to parental antibodies

[00355] The affinity of the antibodies to FGFR2 and FGFR4 following conjugation to SMCC-DM1 was determined using Biacore® technology using a Biacore® T100 instrument (GE Systems) and CM5 sensor chips using similar methodology to that described in example 2 above.

[00356] For the antibodies assessed, similar affinity estimates for binding to human FGFR2 IIIb were obtained for SMCC-DM1 conjugated antibodies relative to parental unconjugated antibodies, suggesting that conjugation does not appreciably impact antibody binding (Table 10).

Table 10. Affinity estimates for unconjugated and SMCC-DM1 conjugated antibodies

Antibody ID	Human FGFR2 IIIb affinity (K _D) (nM)	
	Unconjugated antibody	Antibody-MCC-DM1
10164	2	2.6
10220	17	7.8
10846	3.5	3.8
10918	2.2	2.1
10923	2.2	2.3
11722	4.3	8.8
11723	6.6	5.9
11725	3.3	2.9
12422	5.1	N.D.
12425	2.1	2.3
12433	5.6	4.9
12439	15	N.D.

12931	3.4	N.D.
12944	9.4	N.D.
12947	2.7	N.D.

N.D. = not determined

[00357] In addition, the affinities of several SMCC-DM1 conjugated antibodies to FGFR2 and FGFR4 species orthologues was determined. In these studies, no appreciable differences in affinity were found between the conjugated and unconjugated antibodies (Table 11).

Table 11. Affinities of SMCC-DM1 conjugated antibodies to FGFR2 and FGFR4 species orthologues

Protein	Affinity (K _D) (nM)					
	12433	12433-MCC-DM1	10164	10164-MCC-DM1	12425	12425-MCC-DM1
Human FGFR 2IIIb	5.6	4.9	2	2.6	2.1	2.3
Mouse FGFR2 IIIb	2.2	1.2	10	10	3.2	3.6
Rat FGFR2 IIIb	3.7	2.8	2.8	3.5	3.1	3.5
Cynomolgus monkey FGFR2 IIIb	1.7	1.5	9	3.8	1.6	1.9
Human FGFR4	No binding	N.D.	No binding	N.D.	4.0	4.6
Mouse FGFR4	N.D.	N.D.	N.D.	N.D.	3.6	2.2
Rat FGFR4	N.D.	N.D.	N.D.	N.D.	5.2	4.2
Cynomolgus monkey FGFR4	N.D.	N.D.	N.D.	N.D.	2.3	2.1

N.D. = not determined

Example 6: In vitro activity of ADCs in SNU16 and Kato-III cells

[00358] Following conjugation to the SMCC-DM1 linker-payload, the ability of the antibody drug conjugates (ADCs) to inhibit the proliferation of FGFR2-amplified cell lines, SNU16 (ATCC catalog #CRL-5974) and Kato-III (ATCC catalog # HTB-103) was determined. Briefly, cells were cultured in a tissue culture incubator at 37°C with 5% CO₂ in culture medium as recommended by the supplier. On the day of the assay, cells were washed twice with PBS (Lonza, catalog # 17516C), prior to being treated with 0.25% trypsin-EDTA (Gibco® catalog # 25300) for 5 min and resuspension in the recommended culture medium. Cells were then counted and seeded in 96 well plates (Costar catalog # 3940 or Corning® catalog # 3340) at densities of 2600-3600 cells/well in 100 µl of cell culture medium. A duplicate plate was generated for a day 0 measurement and all plates were incubated in a tissue culture incubator at 37°C with 5% CO₂ overnight. Medium only wells were also generated to act as negative controls. Following this incubation, 50 µl/well of Cell titer Glo® reagent (Promega catalog # G7573) was added to the day 0 plates, which were then shaken gently for 10 min and the resulting luminescence intensity was measured using a Perkin Elmer EnVision® 2101 plate reader. Test ADCs were serially diluted in duplicate to a 2X stock solution in the appropriate cell culture medium and, following the removal of 50 µl of medium from each of rest of the assay plates, 50 µl of 2X serially diluted ADCs were added (final assay concentration 0.2-50 nM DM1 equivalents) prior to incubation in a tissue culture incubator at 37°C with 5% CO₂ for 5 days. Following this incubation period, relative cell viability was determined via the addition of Cell titer Glo® reagent as described above. The effect of the ADCs on cell proliferation was calculated using the average of the duplicates as follows:

$$\% \text{ Inhibition} = (\text{ADC treated} - \text{untreated}) / (\text{untreated} - \text{Day 0}) * 100$$

The % inhibition data was fitted to a 4-parameter logistic equation and IC₅₀ values were determined. Unlike the IgG control ADC, the tested ADCs were found to be potent inhibitors of the proliferation of both Kato-III and SNU16 cells with IC₅₀'s of less than 3 nM (Table 12).

Table 12. Antibody potency as MCC-DM1 ADCs

Antibody ID	Cell proliferation IC ₅₀ (nM)	
	Kato-III	SNU16
10164	1.6	0.5
10220	0.33	<0.2

10846	0.43	<0.2
10918	0.61	<0.2
10923	0.45	<0.2
11722	0.59	<0.2
11723	0.91	<0.2
11725	5.2	5
12422	< 3	< 3
12425	0.15	< 0.2
12433	< 3	< 3
12439	< 3	< 3
12931	< 3	< 3
12944	< 3	< 3
12947	< 3	< 3
IgG control ADC	>50	>25

[00359] The ability of several anti-FGFR antibodies conjugated via the SPDB-DM4 linker-payload was also evaluated. These studies, which were conducted as described above, revealed that the antibodies evaluated were also potent inhibitors of cell proliferation as SPDB-DM4 ADCs, suggesting that their ability to successfully deliver payloads to kill cells is not limited to MCC-DM1. Data for the SNU16 cell line is summarized in Table 13.

Table 13. Antibody potency as SPDB-DM4 ADCs

Antibody ID	Cell proliferation IC50 (nM)
12422	0.07
12425	0.04
12433	0.8
IgG control ADC	>25

Example 7: Assessment of in vivo activity of anti-FGFR2/4 ADCs

[00360] The antitumor activity of 15 anti-FGFR2 or anti-FGFR2/FGFR4 cross-reactive ADCs was evaluated in the gastric, FGFR2 amplified (CN = 31), FGFR2 IIIb isoform expressing SNU16 xenograft tumor model. In two independent studies, female nude mice were implanted subcutaneously with 10×10^6 cells in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the first study eight days post implantation with average tumor volume of 192.9 mm³. After being randomly assigned to one of nine groups (n = 8/group), mice were administered PBS or a single 3 mg/kg intravenous (i.v.) injection of one of the following antibody drug conjugates: 12433-MCC-DM1, 10164-MCC-DM1, 10220-MCC-DM1, 10918-MCC-DM1, 10923-MCC-DM1, 11722-MCC-DM1, 12422-MCC-DM1, or 12425-MCC-DM1. Mice were enrolled in the second study seven days post implantation with average tumor volume of 223.4 mm³. After being randomly assigned to one of nine groups (n = 8/group), mice were administered PBS or a single 3 mg/kg i.v. injection of one of the following antibody drug conjugates: 12433-MCC-DM1, 12947-MCC-DM1, 12931-MCC-DM1, 12944-MCC-DM1, 12439-MCC-DM1, 10846-MCC-DM1, 11725-MCC-DM1, or 11723-MCC-DM1. 12433-MCC-DM1 was included as a bridge between the two studies to facilitate direct comparison of antibody drug conjugates in both studies. Tumors were calipered twice per week. ADCs evaluated in these studies exhibited a broad spectrum of anti-tumor activity 23 to 24 days post dose (ranging from 33.2% T/C to 77.8% regression) (FIG. 4 (A) and (B)).

[00361] The antitumor activity of cleavable disulfide linker based SPDB-DM4 FGFR2 ADCs was evaluated in the SNU16 xenograft tumor model. In these studies, female nude mice were implanted subcutaneously with 10×10^6 cells in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled onto study 11 days post implantation with average tumor volume of 223 mm³. After being randomly assigned to one of five groups (n = 7/group), mice were administered PBS (10 ml/kg), a single 15 mg/kg intravenous injection of 12433-MCC-DM1 or control IgG-MCC-DM1, or a single 5 mg/kg intravenous injection of 12433-SPDB-DM4 or IgG-SPDB-DM4. Tumors were calipered twice per week. Neither control ADCs exhibited antitumor activity in this study (FIG. 4(C)). However, in

contrast, both 12433-MCC-DM1 and 12433-SPDB-DM4 were highly active against the SNU16 xenograft model at the doses administered, suggesting that anti-tumor effects of anti-FGFR ADCs can be obtained with different linkers.

[00362] The pharmacokinetics (PK) of anti-FGFR ADCs was evaluated in SNU16 tumor-bearing mice following a single IV dose of 3 mg/kg. PK samples were collected at 1 h, 24 h, 72 h, 168 h, and 336 h post dose. In all studies, serum concentrations of both “total” antibody and “antibody drug conjugate (ADC)” were measured in all animal species using validated ELISA methods. The “total” fraction refers to the measurement of the antibody with or without the conjugated DM1, whereas the ADC fraction refers to the measurement of DM1 conjugated antibody only (≥ 1 DM1 molecule). In all studies, no appreciable differences were observed between clearance of the ADC and total antibody fractions. A summary of the PK properties of the ADCs is presented in FIG. 4 (D) – (E). When the ADC clearance following a single 3 mg/kg dose in SNU16 tumor bearing mice was compared to the observed anti-tumor effect, it was found that the ADCs with the greatest anti-tumor activity ($> 40\%$ tumor regression) were all cleared at a rate of less than 45 ml/d/kg (FIG. 4 (F)).

Example 8: Assessment of signaling activity of unconjugated and conjugated antibodies in vitro using FGFR2-amplified cell lines

[00363] The ability of the unconjugated and SMCC-DM1 conjugated anti-FGFR2 antibodies to modulate FGFR signaling in FGFR2-amplified cell lines was evaluated. In initial studies, the effect of the 12433, 12425 and 10164 antibodies in SNU16 (ATCC catalog #CRL-5974) cells was determined. Briefly, cells were seeded in RPMI supplemented with 10% FBS in 12 well CellBind™ plates (Costar catalog #3336) and incubated overnight in a tissue culture incubator at 37°C with 5% CO₂. On the day of the experiment, the cell culture medium was aspirated and replaced with either test antibodies or the small molecule FGFR inhibitor, BGJ398, all diluted in RPMI supplemented with 10% FBS at final concentrations of (13-130 nM, antibodies/ADCs; 500 nM, BGJ398). Cells were then incubated in a tissue culture incubator at 37°C with 5% CO₂ for 2 h. Following this incubation, cells were washed twice with cold PBS (Lonza, catalog # 17516C), placed on ice and 300 µl of lysis buffer (CST catalog # 9803 with PhosphoSTOP Roche catalog # 04 906237001) was added. Protein concentration was determined by BCA assay (Pierce catalog # 23228). 60 µg of protein was then resolved by SDS-PAGE, transferred onto nitrocellulose membranes and probed with antibodies to pFRS2 (Cell Signaling

Technology, catalog #3861), total FGFR2 (*e.g.* Santa Cruz Biotechnology, catalog # SC-122 or R&D systems catalog # 6841) and β -actin (Bethyl, catalog # A300-485A) or cytokeratin (Dako catalog # M3515). Results are presented in FIG. 5 (A) and it was found that none of the antibodies, either unconjugated or as MCC-DM1 ADCs were able to modulate pFRS2 signal at 2 h. Further experiments were conducted with the 12425 antibody (4.3 nM) and 12425-MCC-DM1 ADC (4.3 nM antibody, 13 nM DM1 equivalents) in which additional time points (4, 24 and 72 h) were evaluated in both SNU16 and also Kato-III (ATCC catalog # HTB-103) cells. Results are presented in FIG. 5 (B) (SNU16) and 5 (C) (Kato-III). No effects of either the naked antibody or ADC were observed up to 48 h in SNU16 cells or up to 72 h in Kato-III cells. A reduction in both pFRS2 and tFGFR2 signal was observed at 72 h in SNU16 cells relative to cytokeratin following 12425-MCC-DM1 treatment. As described in example 9 below, 12425-MCC-DM1 is a potent inhibitor of SNU16 proliferation at the 72 h timepoint and this decrease likely reflects heterogeneity of FGFR2 expression in the SNU16 cell population.

Example 9: Assessment of ADC and unconjugated antibodies on cellular proliferation

[00364] The ability of the unconjugated and SMCC-DM1 conjugated anti-FGFR antibodies to inhibit the proliferation of a panel of FGFR2-amplified, over-expressing and null cell lines was evaluated. In these studies, cells were cultured in a tissue culture incubator at 37°C with 5% CO₂ in culture medium as recommended by the supplier. On the day of the assay, cells were washed twice with PBS (Lonza, catalog # 17516C), prior to being treated with 0.25% trypsin-EDTA (Gibco® catalog # 25300) for 5 min and resuspension in the recommended culture medium. Cells were then counted and seeded in either 96 or 384 well plates (*e.g.* Costar catalog # 3940, Corning® catalog # 3340, Costar catalog # 3707) at densities of 800-3600 cells/well in 55-100 μ l of cell culture medium. A duplicate plate was generated for a day 0 measurement and all plates were incubated in a tissue culture incubator at 37°C with 5% CO₂ overnight. Medium only wells were also generated to act as negative controls. Following this incubation, 30-50 μ l/well of Cell titer Glo® reagent (Promega catalog # G7573) was added to the day 0 plates, which were then shaken gently for 10 min and the resulting luminescence intensity was measured using a Perkin Elmer EnVision® 2101 plate reader. Test ADCs were serially diluted in duplicate to either a 2X or 10X stock solution in the appropriate cell culture medium. For assays in which a 2X ADC stock solution was used, half of the assay media was removed and replaced with an equal volume of the 2X serially diluted ADCs. Where a 10X ADC stock solution was employed, this was

diluted 1:10 into the assay plates (*e.g.* 5 μ l to 55 μ l) prior to incubation in a tissue culture incubator at 37°C with 5% CO₂ for 5 or 6 days. Final assay concentration of the ADCs ranged from 0.005-100 nM DM1 equivalents. Following this incubation period, relative cell viability was determined via the addition of Cell titer Glo® reagent as described above. The effect of the ADCs on cell proliferation was calculated using the average of the duplicates as follows:

$$\% \text{ Inhibition} = (\text{ADC treated} - \text{untreated}) / (\text{untreated} - \text{Day 0}) * 100$$

[00365] The % inhibition data was fitted to a 4-parameter logistic equation and IC₅₀ values were determined. Representative data for 12425-MCC-DM1 is presented in FIG. 6 (A). This ADC was found to be a potent inhibitor of cell proliferation in both the SNU16 (FIG. 6 (A)) and Kato-III FGFR2-amplified cell lines (FIG. 6 (B)). These cell lines were both confirmed to be sensitive to the maytansinoid payload (L-Me-DM1; free DM1 in FIG. 6 (A) – (C)) but not to a non-targeting ADC (directed to chicken lysozyme) conjugated via SMCC-DM1 at a similar maytansinoid antibody ratio. 12425-MCC-DM1 was also not active against a gastric cancer cell line, NUGC3, that is devoid of appreciable FGFR2 expression but that is sensitive to the maytansinoid payload (FIG. 6 (C)). In addition, the unconjugated antibody, 12425, was found to lack anti-proliferative activity in both SNU16 (FIG. 6 (D)) and Kato-III cells (data not shown). A summary of the anti-proliferative activity of several anti-FGFR-MCC-DM1 ADCs in FGFR2 amplified cell lines, including SNU16, Kato-III, SUM-52, MFM223 and H716 cells (Kunii *et al.*, 2008 Cancer Res 68: 2340-2348; Turner *et al.*, 2010 29: 2013-2023; Mathur *et al.*, 2010. Proceedings of the 101st Annual Meeting of the American Association for Cancer Research, poster # 284) is shown in Table 14. In addition, none of the ADCs were found to be active against a panel of tumor cell lines, including AZ521, CAL-51, KYSE-150, TE-6, SNU-1041, TT, CHL-1, G401 and HEC59.

Table 14. Anti-proliferative activity of several anti-FGFR-MCC-DM1 ADCs in FGFR2 gene amplified & non-amplified cell lines

			<i>In vitro</i> potency (IC ₅₀ , nM free DM1 equivalents)			
Cell Line	Lineage	FGFR2 amplification? (Isoform)	12433-DM1	10164-DM1	12425-DM1	IgG-DM1
SNU16	Gastric	Yes (IIIb)	0.08	0.04	0.03	11
Kato-III	Gastric	Yes (IIIb)	0.28	0.32	0.34	30
SUM-52	Breast	Yes (IIIb)	0.29	0.14	0.17	>30
MFM223	Breast	Yes (IIIb)	24	3.5	1	>30
H716	Colon	Yes (IIIc)	>30	1.7	0.1-0.7	>30
NUGC3	Gastric	No (IIIb)	>30	>30	>30	>30

Example 10: In vivo PK-PD of FGFR ADCs

[00366] Studies were conducted to assess the ability of 12425-MCC-DM1 to modulate pharmacodynamic markers *in vivo*. The goal of these studies was to evaluate the relationship between FGFR2 or FGFR4 expression and G2/M cell cycle arrest. Accumulation of pHH3 positive nuclei, as assessed by immunohistochemistry, was used as a marker of G2/M arrest.

[00367] A rabbit polyclonal antibody produced by immunizing animals with a synthetic phosphopeptide corresponding to residues surrounding Ser10 of human histone H3 was obtained from Cell Signaling Technology (Danvers, MA). Briefly, the IHC protocol included heat and standard exposure to Ventana Cell Conditioning #1 antigen retrieval reagent. The primary antibody was diluted to 1:100 and incubated for 60 min at 37°C. Subsequently, incubation with Ventana OmniMap pre-diluted HRP-conjugated anti-rabbit antibody (Cat # 760-4311) was performed for 4 min.

[00368] To assess PD in the FGFR2 amplified SNU16 xenograft model, female nude mice were implanted subcutaneously with 10x10⁶ cells in a suspension containing 50% phenol red-free

matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Six mice were randomly assigned to receive an i.v. dose of either 12425-MCC-DM1 (10 mg/kg) or PBS (10 ml/kg) once tumors reached between 300 and 500 mm³ (n=3/group). Consistent with the expected mechanism of action of the maytansinoid payload, 12425-MCC-DM1 yielded a marked, time-dependent increase in nuclear pHH3 positivity 24 h post dose relative to PBS treated controls (representative images shown in FIG. 7 (A)). Time dependent changes in cleaved caspase 3 were also evaluated. In these studies, a rabbit polyclonal antibody produced by immunizing animals with a synthetic peptide corresponding to amino-terminal residues adjacent to (Asp175) in human caspase-3 was obtained from Cell Signaling Technology (Danvers, MA). The IHC protocol included No Heat and Standard exposure to Ventana Cell Conditioning #1 antigen retrieval reagent. The primary antibody was diluted to 1:300 and incubated for 60 minutes at room temperature. Subsequently, incubation with Ventana OmniMap prediluted HRP-conjugated anti-rabbit antibody (Cat # 760-4311) was performed for 4 minutes. Similar to pHH3, time dependent changes in cleaved caspase 3 were also observed (FIG. 7(A)). Similar data was also obtained for other anti-FGFR2 ADCs *e.g.* 12433-MCC-DM1.

[00369] To assess ADC specificity, PD was evaluated in the FGFR2/FGFR4 negative NUGC3 xenograft model. In these studies, female nude mice were injected subcutaneously with 1×10^6 cells in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Nine mice were randomly assigned to receive a single intravenous 15 mg/kg dose of 12425-MCC-DM1, control IgG-MCC-DM1 or PBS (10 ml/kg) once tumors reached between 300 and 500 mm³ (n=3/group). 12425-MCC-DM1 failed to modulate pHH3 levels relative to a 15 mg/kg i.v. dose of control IgG-MCC-DM1 in FGFR2 and FGFR4 negative NUGC3 xenografts (representative images shown in FIG. 7(B)). Similar data was also obtained for other anti-FGFR2 ADCs *e.g.* 12433-MCC-DM1.

[00370] Taken together, these data demonstrate that 12425-MCC-DM1 is capable of eliciting robust *in vivo* cellular PD effects that are dependent upon FGFR2 or FGFR4 expression and consistent with the mechanism of action of the maytansinoid payload.

Example 11: In vivo efficacy of anti-FGFR ADCs

[00371] The anti-tumor activity of anti-FGFR2 and/or anti-FGFR2/4 ADCs was evaluated in several tumor xenograft models.

Xenograph models with FGFR2 genomic amplification

[00372] *Model 1:* The antitumor activity of three anti-FGFR2 or anti-FGFR2/FGFR4 dual-targeting ADCs was evaluated in the gastric, FGFR2 amplified (copy number = 31, SNP6.0), FGFR2 IIIc isoform expressing NCI-H716 colorectal xenograft tumor model. Female nude mice were implanted subcutaneously with 5×10^6 cells containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l.

[00373] Mice were enrolled in the study four days post implantation with average tumor volume of 171.7 mm³ (FIG. 8 (A)). After being randomly assigned to one of eight groups (n = 6/group), mice were administered PBS (10 ml/kg), a single i.v. dose of control IgG-MCC-DM1 (15 mg/kg), 12422-MCC-DM1 (5 or 15 mg/kg), 12425-MCC-DM1 (5 or 15 mg/kg), or 10164-MCC-DM1 (5 or 15 mg/kg). Tumors were calipered twice per week. The control IgG-MCC-DM1 was not active at 15 mg/kg. 10164-MCC-DM1 was not active at 5 mg/kg. 10164-MCC-DM1 (15 mg/kg), 12422-MCC-DM1 (5 and 15 mg/kg) and 12425-MCC-DM1 (5 and 15 mg/kg) had exhibited similar activity through 14 d post dose. 12425-MCC-DM1 (5 mg/kg and 15 mg/kg) and 10164-MCC-DM1 (15 mg/kg only) yielded the most durable response through 18 d post dose (23, 21, and 31% T/C, respectively).

[00374] *Model 2:* The dose response antitumor activity of three anti-FGFR2 or anti-FGFR2/FGFR4 ADCs was also evaluated in the, FGFR2 amplified, FGFR2 IIIb isoform expressing MFM223 ER/PR/Her2 negative breast xenograft tumor model. The MFM223 model was established as a fragment based model. One day before fragment implantation, female nude mice were implanted subcutaneously with 0.72 mg, 60 day sustained release 17 β -estradiol pellet (Innovative Research of America) to maintain serum estrogen levels. One day after 17 β -estradiol pellet implantation, xenografts harvested from donor mice were cut into 3 by 3 mm³ fragments subcutaneously implanted into recipient nude female mice. Mice were enrolled in the study 21 days post implantation with an average tumor volume of 208.4 mm³ (FIG. 8 (B)). After being randomly assigned to one of five groups (n = 8/group), mice were administered PBS (10 ml/kg) or a single 10 mg/kg i.v. dose of control IgG-MCC-DM1, 12433-MCC-DM1, 10164-MCC-DM1, or 12425-MCC-DM1. Tumors were calipered twice per week. IgG-MCC-DM1 was not active in

this model. A single 10 mg/kg i.v. dose of 12433-MCC-DM1, 12425-MCC-DM1, and 10164-DM1 yielded 37, 24, and 7% T/C 18 d post administration.

[00375] *Model 3:* The activity of 12425-MCC-DM1 as a single agent was also evaluated in an FGFR2-amplified patient-derived primary gastric tumor xenograft model, CHGA-010 (FGFR2 copy number = 48, (SNP6.0)). In these studies, female nu/nu athymic mice were implanted subcutaneously with 3x3x3mm tumor fragments containing 50% phenol red-free matrigelTM (BD Biosciences) in DMEM. The tumor take rate was > 50% and reached approximately 250 mm³ at 4 weeks post implantation. Following a single dose of 10 mg/kg IV 12425-MCC-DM1, tumor stasis was achieved through approximately 16 to 20 days post-dose (FIG. 8 (C)). Similar single dose data was also obtained for the FGFR2 specific ADC, 12433-MCC-DM1 (data not shown). In addition, a group receiving 10 mg/kg q3w*2 IV maintained roughly tumor stasis through 37 d post initial dose while tumors in the group receiving a single 10 mg/kg dose averaged 1588 mm³ by 37 d post initial dose. These data demonstrate that the CHGA010 xenografts are able to respond to a second dose of 12425-MCC-DM1.

Xenograph models with normal FGFR2 and/or FGFR4 copy number

[00376] The antitumor activity of 12425-MCC-DM1 was also evaluated in twenty three human lung primary tumor xenografts and twenty two human breast primary tumor xenografts with normal FGFR2 copy number and a range of FGFR2 and FGFR4 mRNA expression. Human primary xenograft models were established by subcutaneously implanting human lung primary tumor fragments into female nude mice. The resulting tumor xenografts were passaged *in vivo* by subcutaneous implantation of 3 mm³ fragments into female nude mice. Animals were implanted with tumor fragments and assigned to treatment (untreated or 12425-MCC-DM1, 15 mg/kg i.v. q2w) via rolling enrollment; i.e. once tumors reached approximately 250 mm³ but not necessarily the same time post tumor fragment implantation. One animal per xenograft model was assigned to each of the two treatment arms. Tumors were calipered twice per week and data were analyzed as percent change in tumor volume.

[00377] Eight of twenty three lung primary tumor xenograft models and eight of twenty two breast primary tumor xenograft models responded to 12425-MCC-DM1. The responses ranged from transient tumor growth inhibition to complete regression of the tumor xenograft. An example of one lung primary tumor xenograft model and one breast primary tumor xenograft model that responded to 12425-MCC-DM1 is shown in FIG. 8(D) and 8(E), respectively,

respectively. Taken together, the data demonstrate that *in vivo* growth of human lung and breast primary tumor xenografts with elevated expression of either FGFR2 or FGFR4 mRNA can be inhibited by 12425-MCC-DM1 treatment.

[00378] The dose response antitumor activity the anti-FGFR2/FGFR4 ADC 12425-MCC-DM1 was also evaluated in a breast xenograft model which expresses FGFR2 but has normal copy number of the gene. The model was established as a fragment based xenograft model from a 52 year old female with ductal carcinoma and expanded in female nude mice. Xenografts harvested from donor mice were cut into 3 by 3 mm³ fragments and subcutaneously implanted into recipient nude female mice supplemented with 0.0085mg/ml 17 β -estradiol enriched water to maintain serum estrogen levels and support xenograft growth. Mice were enrolled in the study 26 days post implantation with an average tumor volume of 198 mm³. After being randomly assigned to one of three groups (n = 6/group), mice were administered a single 15 mg/kg i.v. dose of control IgG-MCC-DM1 or a single i.v. dose of 12425-MCC-DM1 at 15 or 5 mg/kg (FIG. 8(F)). Tumors were calipered twice per week. A single 15 mg/kg i.v. dose of 12425-MCC-DM1 yielded 6.5% T/C while the 5 mg/kg dose was inactive. These data support the utility of 12425-MCC-DM1 in FGFR2 expressing tumors without gene amplification.

[00379] Taken together, these models support the utility of 12425-MCC-DM1 in tumors that express FGFR2 at the cell surface either in the presence or absence of FGFR2 gene amplification.

Example 12: Improved anti-tumor activity of FGFR ADC with small molecule FGFR inhibitor, BGJ398 (also known as 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea)

[00380] Anti-tumor activity of FGFR2/4 antibody drug conjugate 12425-MCC-DM1 alone and in combination with the FGFR small molecule tyrosine kinase inhibitor, 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea (Guagnano *et al.*, 2011 J Med Chem 54: 7066-7083) was evaluated in the FGFR2-amplified CHGA119 patient-derived primary gastric tumor xenograft model (FGFR2 copy number = 22 (SNP6.0)). In these studies, CHGA119 tumor fragments in size of 2mm x 2mm x 2mm were implanted subcutaneously (s.c.) into female nu/nu athymic mice. Forty days after implantation, mice carrying CHGA119 tumors (n=8, average 251 mm³; range: 104-382 mm³) were treated with vehicle (50% PEG300 in acetic acid/acetate buffer) orally via gavage (pH4.6, 10 ml/kg, p.o., qd), control IgG-3207-DM1 (10 mg/kg, i.v., q2wk), 12425-MCC-DM1 (10 mg/kg, i.v., q2wk), 3-(2,6-

Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea phosphate (10 mg/kg, p.o., qd), or the combination of 12425-MCC-DM1 and 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea phosphate, respectively. Tumors were calipered twice a week. 12425-MCC-DM1 and 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea phosphate each as single agent resulted in tumor stasis or partial response (T/C=4% or 33%, respectively, $p<0.05$), while the treatment in combination of 12425-MCC-DM1 and 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea phosphate induced near complete tumor regression after 5 weeks of treatment (Regression=-94%, $p<0.05$) (see FIG. 9 and Table 15). Less than 10% body weight loss was observed following either the single agent or combination treatments (Table 15). These data suggest that combining FGFR ADCs with small molecule inhibitors of FGFR signaling (*e.g.* 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea phosphate, TKI258, ponatinib, AZD4547) can improve anti-tumor responses.

Table 15. Effect of anti-FGFR agents on tumor and host parameters in CHGA119-tumor bearing mice

Treatment	Tumor response			Host response		
	T/C (%)	Regression (%)	Mean tumor volume change (mm ³ ± SEM)	Mean body weight change (g ± SEM)	%body weight change (mean ± SEM)	Survival (survivors/total)
Vehicle	100	-	1168±204	-1.3±0.3	-5.7±1.4	8/8
IgG-MCC-DM1	111	-	1301±243	-1.7±0.6	-7.6±2.7	8/8
12425-MCC-DM1	4	-	43±35	0.6±0.2	2.5±1.0	8/8
BGJ398 phosphate	33	-	386±169	-0.8±0.7	-3.7±3	8/8

12425-MCC-DM1+BGI398	-	94	-223±32	-3.7±3.8	-4.3±3.8	7/8
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Example 13: Effect of dose fractionation on in vivo efficacy of FGFR ADC

[00381] To better understand the role 12425-MCC-DM1 $C_{\max}$ (peak exposure) or C_{avg} (average exposure over a dosing interval) play in its antitumor activity, a time-dose fractionation experiment was conducted using the SNU16 xenograft model. A range of dose schedule intervals and dose levels yielding a predicted fixed total exposure over a fixed treatment duration were studied. At certain dose levels, FGFR2 ADCs exhibit nonlinear PK due to TMDD (see example 19). Therefore, in order to maintain a fixed total exposure across all dose schedule intervals and dose levels, PK modeling predicted that a larger total dose of 12425-MCC-DM1 would be required when administered at a lower, more frequent dose schedule as TMDD would be more pronounced at lower doses. PK modeling predicted that 12425-MCC-DM1 administered at 3 mg/kg q3w*2, 2.5 mg/kg q2w*3, 1.5 mg/kg qw*6, and 0.7 mg/kg q3d*14 would achieve similar total exposure.

[00382] Female nude mice were implanted subcutaneously with 10×10^6 cells in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μl . Mice were enrolled in the first study ten days post implantation with average tumor volume of 181.7 mm^3 .

[00383] To assess PK parameters, serum was collected via tail nick or retro-orbital bleeds and analyzed via ELISA. The total antibody PK assay measures total antibody concentration, with/without DM1 by colorimetric ELISA. Plates are coated with anti-human IgG (Fc specific), and detection is with donkey anti-human IgG-HRP before being read on an appropriate plate-reader. The conjugate PK assay measures antibody that is bound to at least 1 DM1 molecule by colorimetric ELISA. In this format, plates are coated with anti-DM1 antibody, and detected with donkey anti-human IgG-HRP. Serum was collected at the following time points post initial dose: 3 mg/kg q3w*2 (2, 6, 24, 48, 96, 168, 240, 336, 503 h); 2.5 mg/kg q2w*3 (2, 6, 24, 48, 96, 168, 240, 335, 503 h); 1.5 mg/kg qw*6 (2, 6, 24, 48, 96, 167, 335, 503, 671, 839 h); 0.7 mg/kg q3d*14 (2, 6, 24, 48, 71, 143, 215, 287, 359, 431, 503, 575, 647, 719, 791, 863, 935 h).

[00384] After being randomly assigned to one of five groups ($n = 7/\text{group}$), mice were administered PBS (10 ml/kg) or 12425-MCC-DM1 administered i.v. at 3 mg/kg q3w*2, 2.5 mg/kg q2w*3, 1.5 mg/kg qw*6, or 0.7 mg/kg q3d*14. As anticipated, $C_{\max}$ varied across

treatment regimens while C_{avg} was static (Table 16). All treatment regimens were active and yielded similar tumor growth inhibition suggesting that average exposure of 12425-MCC-DM1 is the primary driver of antitumor activity (FIG. 10). These findings indicate that a variety of dose schedules could be employed in the clinic without compromising efficacy.

Table 16. PK parameters following administration of different doses and schedules of 12425-MCC-DM1 to SNU16 tumor-bearing mice

Dosing Regimen	<u>C_{max}[*], ug/ml</u>			<u>C_{avg}_{Tau}, ug/ml</u>		
	Total dose, mg	Intact ADC	t	Total	Intact ADC	Tau, h
3 mg/kg, iv, q3wx2	6	24.5	25.1	2.19	2.29	503
2.5 mg/kg, iv, q2wx3	7.5	21.2	21.6	2.59	2.30	335
1.5 mg/kg, iv, qwx6	9	11.0	11.0	2.56	2.50	167
0.72 mg/kg, iv, q3dx14	10	4.0	4.2	1.94	1.63	72

Example 14: Evaluation of ADCC activity in vitro and in vivo

[00385] The ability of the unconjugated anti-FGFR2 antibodies (12433, 10164, 12425, N297A_12425 (an ADCC-depleted variant (Bolt *et al.*, 2003 Eur J Immunol 23: 403-411))) to mediate antibody dependent cellular cytotoxicity (ADCC) was determined versus Kato-III cells (target cells; ATCC catalog # HTB-103) in co-incubation with NK3.3 cells (killer cells or effector cells; kindly provided by Jacky Kornbluth from San Louis University). In brief, Kato-III cells were stained with Calcein acetoxymethyl ester (Calcein-AM; Sigma-Aldrich catalog # 17783-5MG), washed twice, pipetted into a 96-well microtiterplate (96 well, U-bottomed, clear plastic; Corning® Costar, catalog # 650 160) at a concentration of 5000 cells per well and pre-incubated for 10 min with a serial dilution of the above mentioned antibodies and proteins (from 50,000 to 0.003 μ g per ml) before adding the effector cells. In order to calculate the antibody specific lysis of the target cells, a parallel incubation of target cells only without antibody or effector cells served as a baseline and negative control, whereas the positive control or maximal lysis or

hundred percent specific lysis was determined by lysis of target cells only with a 1% Triton-XTM 100 solution. Following a co-incubation of target and effector cells in a ratio of one to five, the microtiterplate was centrifuged and an aliquot of the supernatant fluid was transferred to another microtiterplate (96 well, flat-bottomed, black with clear bottom; Corning® Costar, catalog # 3904) and the concentration of free Calcein in solution was determined with a fluorescence counter (VictorTM 3 multilabel counter, Perkin Elmer). Results are presented in FIG. 11 (A) and it was observed that all tested antibodies mediated ADCC to a varying extent. The N297A_12425, carrying no glycosylation in the Fc-part of the antibody and thus lacking the binding to the CD16a receptor of the effector cells, did not mediate any ADCC and served as a specific negative control. The constant and protein-concentration independent killing of target cells is due to a unspecific background, natural killing activity of the NK3.3 cells against the Kato-III cells. The antibody 12433 mediated the highest specific lysis of target cells with about sixty percent (signal deduced the background lysis of Kato-III cells by NK3.3 cells), whereas the antibodies 12425 and 10164 reached a specific lysis of about fifty-five percent (signal deduced the background lysis of Kato-III cells by NK3.3 cells). The potency (the concentration at the half maximal killing of each dose response curve; effective concentration 50, EC₅₀) of all three molecules was similar. In a similar series of experiments, the ability of the naked antibody 12425 to induce ADCC was compared 12425-MCC-DM1. No differences between the naked 12425 and 12425-MCC-DM1 were observed (data not shown) suggesting that DM1 conjugation does not appreciably impair the ability of the naked antibody to induce ADCC.

[00386] In additional studies, the ability of the unconjugated anti-FGFR2 antibodies to bind the complement factor C1q was evaluated. The binding of the complement factor C1q is the initial step leading to complement dependent cytotoxicity (CDC) and cell lysis *in vivo*. In these studies the surface of the wells of 96-well microtiterplates (Nunc® ,Maxisorp®, catalog # 439454) were coated with serial dilutions of the antibodies 12433, 12425, N297A_12425 and 10164 over night at 4°C in the dark (all in a concentration range from 25 to 0.02 µg/ml). The commercially available antibody, Rituximab served as a positive control. In order to monitor the amount of bound antibody analyte on the microtiterplate, this procedure was performed twice per plate utilizing the second dilution (in triplicate) for determining the coating efficacy of the analyte to the plate. C1q binding was quantified by adding a constant concentration of C1q (Sigma; Complement component C1q catalog # C1740-1mg) and detected using a polyclonal goat-anti-

human C1q antibody conjugated to Horse Radish Peroxidase HRP (AbD Serotec; Sheep Anti-Human C1q:HRP; Catalogue number 2221-5004P). The control of the coating efficiency was determined in the second dilution series with a peroxidase-conjugated goat-anti-human IgG antibody fragment (Jackson ImmunoResearch; catalog # 109-036-003; AffiniPure F(ab'2) fragment Goat Anti-Human IgG (H+L)). Both were visualized using the TMB substrate (TMB Peroxidase EIA Substrate Kit; Bio-Rad; catalog #172-1067) with subsequent measurement of optical density at 450 nm with a UV-Vis Spectroscope (Molecular Devices; Spectramax®Pro 340). Results are presented in FIG. 11 (B). The maximal amount of C1q protein bound to the tested antibodies was the same for all analytes. The amount of antibody that was bound to the microtiterplate was the same for all analytes except for the 10164 antibody, which reached only around 50% of the other molecules. The potency (the concentration at the half maximal binding of each dose response curve; effective concentration 50, EC_{50}) of 12433, 10164 and Rituximab was about the same. The EC_{50} of the 12425 antibody was a factor of 3 higher and the EC_{50} of the N297A_12425 was a factor of about 8 higher. Given the result that 10164 did not coat equally well to the microtiterplate as the other antibodies did, the observed result in C1q binding implicates an approximate double capacity of 10164 in binding to the C1q protein.

[00387] Further to the assessment of C1q binding, the ability of unconjugated anti-FGFR2 antibodies 12433, 10164 12425 and N297A_12425 to trigger complement dependent cytotoxicity (CDC) was evaluated. Kato-III cells (target cells; ATCC catalogues number HTB-103) were equally distributed into the wells of a 96-well microtiterplate (Costar; sterile, white, 96-well flat bottomed cell culture plates, catalog # 3610) at a concentration of 10000 cells per well and pre-incubated for 10 min with a serial dilution of the above mentioned antibodies before adding the effector reagent. The effector reagent used in this study was rabbit complement (PelFreez; catalog # 31060-1), which was pipetted onto the Kato-III cells at a final dilution of one to six. Following a two hour incubation in a humidified cell culture incubator at 37°C, the microtiterplate was centrifuged, supernatant was discarded and the cell pellet was dissolved in reconstituted CellTiterGlo® (Promega; CellTiterGlo® Luminescence Kit, catalog # G7572). Luminescence was quantified in a multilabel reader (Perkin Elmer; Victor™ 3). Results are presented in FIG. 11 (C) for all tested antibodies. No evidence of CDC was observed for the tested antibodies (FIG. 11 (C)) or additional controls (commercially available antibodies Erbitux® & Herceptin®, data not shown).

[00388] To assess the role of ADCC in 12425-MCC-DM1's *in vivo* activity, female nude mice were implanted subcutaneously with 10×10^6 cells in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study seven days post implantation with average tumor volume of 201.4 mm³. After being randomly assigned to one of five groups (n = 6/group), mice received a 10 mg/kg i.v. dose of naked control IgG, naked N297A_12425, naked 12425, N297A_12425-MCC-DM1, or 12425-MCC-DM1. Tumors were calipered twice per week (FIG. 11 (D)). The naked, ADCC-depleted 12425 variant (naked N297A_12425) exhibited less antitumor activity than the ADCC competent parental 12425 (61 and 13% T/C, respectively) at 25 d post dose. These data suggest that the parental 12425's effector cell function may play a role in the activity of the naked antibody's *in vivo* antitumor activity. The ADCC competent 12425-MCC-DM1 and ADCC depleted N297A_12425-MCC-DM1 exhibited similar anti-tumor activity at 25 d post dose (45 and 32% regression, respectively). These data suggest that while ADCC activity is expendable, that the maytansinoid payload is both required and sufficient for 12425-MCC-DM1's robust *in vivo* antitumor activity.

Example 15: Evaluation of anti-FGFR antibodies and ADCs to modulate signaling in FGFR4-overexpressing cell lines

[00389] The ability of the unconjugated and conjugated anti-FGFR antibodies to modulate FGFR signaling in FGFR4-overexpressing cell lines was evaluated. In initial studies, the effect of the 10164 (FGFR2 specific), 12433 (FGFR2 specific), 12425 (FGFR2/4 cross-reactive) and associated MCC-DM1 conjugates in MDA-MB453 (ATCC catalog # HTB-131) cells was determined. Briefly, cells were seeded in RMPI supplemented with 10% FBS in CellBindTM 12 well plates (Costar, catalog # 3336) and incubated overnight in a tissue culture incubator at 37°C with 5% CO₂. On the day of the experiment, the cell culture medium was aspirated and replaced with either test antibodies or the small molecule FGFR inhibitor, BGJ398, all diluted in RPMI supplemented with 10% FBS at final concentrations of (13-130 nM, antibodies/ADCs; 500 nM, BGJ398). Cells were then incubated in a tissue culture incubator at 37°C with 5% CO₂ for 2 h. Following this incubation, cells were washed twice with cold PBS (Lonza, catalog # 17516C), placed on ice and 300 μ l of lysis buffer (Cell Signaling Technology, catalog # 9803 with PhosphoSTOP (Roche, catalog # 04 906237001) was added. Protein concentration was determined by BCA assay (Pierce, catalog # 23228). Protein concentration was determined by

BCA assay (Pierce). 60 µg of protein was then resolved by SDS-PAGE, transferred onto nitrocellulose membranes and probed with antibodies to pFRS2 (Cell Signaling Technology, catalog #3864), total FGFR4 (R&D Systems, catalog #8652) and β-actin (Bethyl, catalog # A300-485A) or cytokeratin (Dako, catalog # M3515). Results are presented in FIG. 12 (A) and it was found that none of the antibodies, either unconjugated or as MCC-DM1 ADCs were able to modulate pFRS2 signal at 2 h. Further experiments were conducted with the 12425 antibody 4.3 nM and 12425-MCC-DM1 ADC (4.3 nM antibody, 13 nM DM1 equivalents) in which additional time points (4, 24 and 72 h) were evaluated. Results are presented in FIG. 12 (B). Similar data was also obtained in the RH4 rhabdomyosarcoma cell line.

Example 16: Evaluation of anti-proliferative effect of anti-FGFR antibodies and ADCs in FGFR4-overexpressing cell lines

[00390] The ability of the unconjugated and SMCC-DM1 conjugated anti-FGFR antibodies to inhibit the proliferation of a panel of FGFR4 over-expressing cell lines was evaluated using a similar methodology as employed in example 9 described above.

[00391] Consistent with its cross-reactivity to FGFR4, it was found that 12425-MCC-DM1 was a potent inhibitor of the growth of MDA-MB453 cells, which over-express a constitutively active mutant form of FGFR4 (Roidl *et al.*, 2010, Oncogene, 29, 1543-1552). FGFR4 expression was confirmed by FACS analysis using an anti-FGFR4 antibody (Biolegend catalog # 324306; FIG. 13 (A)). 12425 demonstrated similar binding properties to the Biolegend antibody not only in MDA-MB453, but also in other FGFR4-overexpressing cell lines (data not shown). This effect was specific to the ADC, as the unconjugated 12425 antibody was inactive (FIG. 13 (A)). Furthermore, no activity was observed following administration of an FGFR2 specific antibody (10164) or ADC (10164-MCC-DM1), suggesting that the effect is driven by FGFR4 expression (FIG. 13 (A)). This is consistent with the lack of detectable FGFR2 expression on these cells as assessed by FACS (data not shown). A similar effect of 12425-MCC-DM1 and 12425 was also observed in the FGFR4 overexpressing rhabdomyosarcoma cell lines, RH4 (FIG. 13 (B)) and JR (FIG. 13 (C)). When extended to additional cell lines, activity of 12425-MCC-DM1 was observed in a subset of FGFR4 overexpressing breast and rhabdomyosarcoma cell lines (FIG. 13 (D)).

Example 17: In vivo PK-PD of FGFR ADCs and unconjugated antibodies in FGFR4-overexpressing tumors

[00392] To assess PD modulation in the FGFR4 positive MDA-MB-453 xenograft model, female NSG mice were utilized. One day before cell implantation, female NSG mice were implanted subcutaneously with a 0.36 mg, 90 day sustained release 17 β -estradiol pellet (Innovative Research of America). One day after 17 β -estradiol pellet implantation, 5 x10⁶ cells were injected subcutaneously in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Six mice were randomly assigned to receive a single intravenous 15 mg/kg dose of either 12425-MCC-DM1 or control IgG-MCC-DM1 once tumors reached between 300 and 500 mm³ (n=3/group). 12425-MCC-DM1 yielded a marked increase in nuclear pHH3 positivity 96 h post dose relative to control mice receiving IgG-MCC-DM1 (representative images shown in FIG. 14 (A)).

[00393] To assess the time course of PD modulation in the FGFR4 positive MDA-MB-453 xenograft model, female NSG mice were utilized. One day before cell implantation, female NSG mice were implanted subcutaneously with a 0.36 mg, 90 day sustained release 17 β -estradiol pellet (Innovative Research of America). One day after 17 β -estradiol pellet implantation, 5 x10⁶ cells were injected subcutaneously in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Mice were randomly assigned to receive a single intravenous 15 mg/kg dose of either 12425-MCC-DM1 (three mice per group were collected 24, 48, 72, 96, and 168 hours post treatment administration) or PBS (10 ml/kg). Tumor volumes ranged between 300 and 500 mm³ at randomization. Consistent with the expected mechanism of action of the maytansinoid payload, 12425-MCC-DM1 yielded a marked, time-dependent increase in nuclear pHH3 positivity that peaked around 72-96 h h post dose relative to PBS treated controls. A peak in cleaved caspase 3 immunoreactivity occurred around 96 and 168 h post dose. Representative images shown in FIG. 14 (B).

[00394] A portion of alveolar rhabdomyosarcomas, including the RH4 cell line, harbor Pax 3/7-FOXO1A translocations which result in elevated FGFR4 mRNA and protein expression. To assess PD modulation in the RH4 xenograft model, female nude mice were implanted subcutaneously with 10x10⁶ cells in a suspension containing 50% phenol red-free matrigel (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Six mice were randomly assigned to receive a single intravenous 15

mg/kg dose of either 12425-MCC-DM1, control IgG-MCC-DM1, or PBS (10 ml/kg) once tumors reached between 500 and 700 mm³ (n=3/groups). 12425-MCC-DM1 yielded a marked increase in nuclear pHH3 positivity 96 h post dose relative to control mice receiving IgG-MCC-DM1 (representative images shown in FIG. 14 (C)). These findings demonstrate that 12425-MCC-DM1 causes G2/M cell cycle arrest in this FGFR4 positive xenograft model.

Example 18: In vivo efficacy of anti-FGFR ADCs in FGFR4-overexpressing tumors

[00395] The dose response antitumor activity of 12425-MCC-DM1 was evaluated in the FGFR4 positive MDA-MB-453 breast cancer xenograft model. One day before cell implantation, female NSG mice were implanted subcutaneously with a 0.36 mg, 90 day sustained release 17 β -estradiol pellet (Innovative Research of America). One day after 17 β -estradiol pellet implantation, 5 x10⁶ cells were injected subcutaneously in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study 12 days post implantation with average tumor volume of 197.9 mm³. After being randomly assigned to one of five groups (n = 5/group), mice were administered i.v. PBS (10 ml/kg), control IgG-MCC-DM1 (15 mg/kg), unconjugated 12425 IgG (15 mg/kg), or 12425-MCC-DM1 (5, 10, or 15 mg/kg) (FIG. 15 (A)). Control IgG-MCC-DM1 was not active in this model. A single dose of 15 mg/kg naked IgG 12425 or 5 mg/kg 12425-MCC-DM1 exhibited similar activity 29 d post dose (25 and 24% T/C, respectively). 12425-MCC-DM1 administered at both 10 and 15 mg/kg yielded tumor regression (72 and 92% regression, respectively).

[00396] The dose response antitumor activity of 12425-MCC-DM1 was evaluated in the FGFR4 positive, Pax 3/7-FOXO1A positive RH4 xenograft model. Female nude mice were implanted subcutaneously with 10x10⁶ cells in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study 11 days post implantation with average tumor volume of 200.8 mm³ (FIG. 15 (B)). After being randomly assigned to one of five groups (n = 8/group), mice were administered i.v. PBS (10 ml/kg), control IgG-MCC-DM1 (15 mg/kg), unconjugated 12425 IgG (15 mg/kg), or 12425-MCC-DM1 (5, 10, or 15 mg/kg). Control IgG-MCC-DM1 was not active in this model. Similarly, a single 15 mg/kg dose of naked IgG 12425 exhibited limited activity. 12425-MCC-DM1 exhibited anti-tumor activity as a single dose of 5, 10, and 15 mg/kg (36, 24, 26% T/C respectively). These findings

confirm that the Pax 3/7-FOXO1A translocation results in sufficient FGFR4 expression to render the RH4 cell line sensitive to 12425-MCC-DM1. Thus, 12425-MCC-DM1 could be used for treating patients with Pax 3/7-FOXO1A translocation positive rhabdomyosarcomas.

Example 19: Evaluation of pharmacokinetics of anti-FGFR ADCs in mouse, rat and cynomolgus monkey

[00397] The pharmacokinetics (PK) of anti-FGFR ADCs was evaluated in tumor- and non-tumor-bearing mice at several dose levels ranging from 1-15 mg/kg, in non-tumor bearing rats at 1, 5 and 45 mg/kg and in cynomolgus monkey at 30 mg/kg. In all studies, serum concentrations of both “total” antibody and “antibody drug conjugate (ADC)” were measured in all animal species using validated ELISA methods. The “total” fraction refers to the measurement of the antibody with or without the conjugated DM1, whereas the ADC fraction refers to the measurement of DM1 conjugated antibody only (≥ 1 DM1 molecule). In all studies, no appreciable differences were observed between clearance of the ADC and total antibody fractions.

[00398] 12425-MCC-DM1 PK was investigated in SNU16 tumor-bearing mice following a single IV dose of 3 mg/kg. PK samples were collected at 1 h, 24 h, 72 h, 168 h, and 336 h post dose and the serum concentrations of the total and ADC fraction were super-imposable at this dose (FIG. 16 (A)). 12425-MCC-DM1 half-life was about 1.5 days in the presence of the SNU16 xenograft.

[00399] 12425-MCC-DM1 PK was also investigated in non-tumor bearing mice following a single IV dose of 1, 5, 10 or 15 mg/kg. PK samples were collected at 1 h, 6 h, 24 h, 72 h, 96 h, 168 h, 240 h, 336 h, and 540 h post dose. The serum concentrations of the total and ADC fraction were super-imposable at these doses (FIG. 16 (B)) and PK properties appeared similar in non-tumor bearing relative to tumor bearing animals.

[00400] The PK of 12433-MCC-DM1 and 10164-MCC-DM1 in SNU16 tumor-bearing mice was also determined. The overall PK profile of these ADCs was similar to that of 12425-MCC-DM1 and the data obtained is summarized in Table 17.

Table 17: Pharmacokinetic properties of 12433-, 12425- and 10164-MCC-DM1 in mice at 3 mg/kg dose

Ab	C _{max} (ug/mL)	CL (mL/h/kg)	V(mL/kg)	t _{1/2} (h)
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	TOTAL	ADC	TOTAL	ADC	TOTAL	ADC	TOTAL	ADC
12425-MCC-DM1	45.0	50.4	1.85	1.72	97.0	89.5	36.4	36.1
12433-MCC-DM1	52.4	42.7	1.61	1.95	101.0	122.9	43.5	43.8
10164-MCC-DM1	42.8	37.5	1.18	1.28	101.4	117.5	59.4	64.0

[00401] 12425-MCC-DM1 PK was investigated in non-tumor bearing rats at two dose levels; 1 and 5 mg/kg administered IV. PK samples were collected at 0.5 h, 1 h, 2 h, 6 h, 8 h and on day 1, 2, 4, 8, 11, 14, 21 and 35. The concentration-time profiles for the “total” and “ADC” species were near superimposable at both dose levels (FIG. 16 (C)). The terminal elimination phase was slightly steeper in the 1 mg/kg dose group, and this could be as a result of target-mediated drug disposition (TMD) at later time-points. Similarly, clearance values for the total and ADC fractions were 0.757 ± 0.088 mL/h/kg and 0.813 ± 0.041 mL/h/kg, respectively at the 1 mg/kg dose, while slightly lower values of 0.623 ± 0.027 mL/h/kg and 0.607 ± 0.019 mL/h/kg, respectively were noted at the 5 mg/kg dose. 12425-MCC-DM1 ADC species have a terminal half-life of around 3.9 days at the 5 mg/kg dose level. In additional studies, rat PK was also determined at 5 mg/kg and 45mg/kg dose levels. Half-life at the 5 mg/kg dose was similar to the values noted above, while at the 45 mg/kg dose the half-life was 163.9 h, 121.3 h (~5 days) for the total and ADC fractions, respectively and these values were similar to observations for a non-FGFR2/4 cross-reactive control ADC, suggesting saturation of any possible TMD clearance pathways.

[00402] 12433-MCC-DM1 and 10164-MCC-DM1 PK was also determined in similar studies and the PK parameters obtained are presented in Table 18. It was found that at the dose levels employed, similar estimates of total and ADC half-life and clearance were obtained for all 3 ADCs tested.

Table 18: Pharmacokinetic properties of 12433-, 12425- and 10164-MCC-DM1 in rats

Dose (mg/kg)	Ab	t1/2 (h)		AUCinf (ug.h/mL)		CL (mL/h/kg)	
		TOTAL	ADC	TOTAL	ADC	TOTAL	ADC
5	12433-MCC-DM1	71.1	59.0	388.8	374.0	0.536	0.557
45	12433-MCC-DM1	176.7	131.9	4399.3	3929.8	0.426	0.477
5	12425-MCC-DM1	77.0	64.0	12853	12874	0.389	0.388
45	12425-MCC-DM1	163.9	121.3	103265	103647	0.436	0.434
5	10164-MCC-DM1	88.0	78.0	12677	14644	0.394	0.341
45	10164-MCC-DM1	140.1	110.3	139842	150433	0.322	0.299

[00403] 12425-MCC-DM1 PK was investigated following administration of a single 30 mg/kg IV dose to a group of three cynomolgus monkeys (1 male, 2 female). Serum samples were collected on Day 1 - 0 h (prior to dosing), and at 0.25 h, 2 h, 4 h, 6 h, 24 h, 48 h, 72 h, 168 h and 240 h post dose. The maximum exposure to 12425-MCC-DM1 was observed immediately following IV injection, i.e. at 0.25 h, which was the first sampling time-point. The time course was characterized by a long elimination phase, and the profiles for the total and ADC fractions were superimposable over the sampling time course (FIG. 16 (D)). The terminal half-life was around 6-7 days.

[00404] 10164-MCC-DM1 PK was also investigated following administration of a single 30 mg/kg IV dose to a group of three cynomolgus monkeys. The time course for this reagent was also characterized by a long elimination phase (terminal half-life of 6-7 days) and PK parameters of both 10164-MCC-DM1 and 12425-MCC-DM1 are presented in Table 19.

Table 19. Pharmacokinetic properties of 12425- and 10164-MCC-DM1 in cynomolgus monkey following a 30 mg/kg IV dose

Test article		Cmax (µg/mL)	AUC0-240h (µg.h/mL)	AUC0-inf (µg.h/mL)	CL (mL/h/kg)	t1/2 (h)
12425	Total	785.6 ± 72.2	64612 ± 6477	99979 ± 12060	0.303 ± 0.038	166.5 ± 36.4
	ADC	780.6 ± 84.2	66656 ± 7577	99113 ± 16895	0.310 ± 0.052	151 ± 22.3
10164	Total	1032 ± 423	79535 ± 6506	124946 ± 11687	0.242 ± 0.0237	175.1 ± 44.1
	ADC	909.5 ± 224.3	81150 ± 4658	122061 ± 8615	0.247 ± 0.018	158.1 ± 33

Example 20: Assessment of the activity of sequence-modified anti-FGFR antibodies and ADCs in vitro and in vivo

[00405] It is recognized that potential structural liabilities can exist in the sequences of therapeutic antibodies that can affect the heterogeneity of the final protein and may impact, for example, antibody manufacturability and immunogenicity. Such liabilities can include glycosylation sites, un-paired cysteines, potential deamidation sites etc. To reduce the risk of such potential liabilities, mutations can be introduced to remove one or more of these liabilities. For example, potential deamidation sites can be replaced either alone or in conjunction with other structural changes. Examples of potential deamidation sites include DG in heavy chain CDR2 of 10164 and 12425 (see Table 1). These residues can be mutated to other appropriate amino acids. By way of non-limiting example, the aspartic acid (D) in 10164 HCDR2 can be mutated to glutamic acid (E) or threonine (T). In 12425, the HCDR2 glycine (G) can be mutated to another amino acid, such as alanine (A).

[00406] It should also be appreciated that where an antibody differs from its respective germline sequence at the amino acid level, the antibody can be mutated back to its germline sequence. Such corrective mutations can occur at one or more positions and be generated using standard molecular biology techniques or by gene synthesis. By way of non-limiting example, the heavy chain sequence of 12425 heavy chain (SEQ ID NO:9, see Table 1) differs from the corresponding germline sequence by an E to a Q at position 1, and the light chain (SEQ ID No:

17) differs from the corresponding germline by a T for a V at position 85. Thus the amino acids in 12425 can be modified at any or all of these sites.

[00407] The chemistry employed to generate the antibody drug conjugates described in this application relies on conjugation to lysine (K) residues in the antibody sequence. Where these lysine residues exist within regions involved in epitope recognition, for example, in CDR regions, if significant conjugation occurs at these sites, the ability of the antibody drug conjugate to bind to its intended target could be altered. To mitigate this potential risk, such lysine residues can be mutated to alternative appropriate amino acids, such as arginine, asparagine or glutamine. One lysine is present in both the heavy chain CDR2 and CDR3 regions of 12425. Either one or more of these lysines can be mutated to alternative residues, such as arginine, asparagine or glutamine.

[00408] Several of the changes described above were introduced into the 12425 antibody to yield the antibody 20562 (Table 1). This antibody was compared to 12425 for its ability to bind to FGFR2, its ability to inhibit the proliferation of FGFR2-amplified cell lines, such as SNU16 and to cause an anti-tumor effect against SNU16 cells *in vivo*. The affinity of 20562 to human FGFR2 IIIb and FGFR4 was found to be similar to that of 12425 under similar assay conditions (20562 FGFR2 IIIb affinity estimate: 9 nM; human FGFR4 affinity estimate: 3.3 nM). Employing the methodology described in example 6, similar to 12425-MCC-DM1, 20562-MCC-DM1 was also a potent inhibitor of the proliferation of SNU16 cells *in vitro* (FIG. 17 (A)). To assess the potency of 20437-MCC-DM1 *in vivo*, female nude mice were implanted subcutaneously with 10×10^6 cells in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study seven days post implantation with an average tumor volume of 217.1 mm³. After being randomly assigned to one of five groups (n = 6/group), mice received PBS (10 ml/kg) or a 3 mg/kg i.v. dose of 12425-MCC-DM1 or 20562-MCC-DM1. Tumors were calipered twice per week (FIG. 17(B)). Both 12425-MCC-DM1 and its variant 20562-MCC-DM1 had similar activity *in vivo* against the FGFR2 amplified SNU16 xenograft. These data suggest that it is possible to remove the DG site of the parent antibody without impacting *in vivo* activity as an ADC.

Example 21: Generation and characterization of affinity matured FGFR ADCs

[00409] To evaluate the effect of affinity on the biological activity of selected anti-FGFR antibodies, affinity optimization was performed on the 12433, 10164 and 12425 clones. In these

studies, the L-CDR3 and H-CDR2 regions were optimized in parallel by cassette mutagenesis using trinucleotide directed mutagenesis (Virnekas *et al.*, 1994 Nucleic Acids Research 22: 5600-5607), while the framework regions were kept constant. Prior to cloning for affinity maturation, parental Fab fragments were transferred from the corresponding expression vector pM[®]x11 into the CysDisplay[™] vector pMORPH[®]30 via *XbaI/EcoRI*.

[00410] For optimizing L-CDR3 of parental Fab fragments, the L-CDR3 of the binder was removed and replaced by a repertoire of diversified L-CDR3s. For each parental Fab, a second library set with diversified H-CDR2 has been generated. For each maturation library (LCDR3 and HCDR2), antibody-displaying phages were prepared and phage titers were determined by spot-titration. Amplification of the library was performed as described elsewhere (Rauchenberger *et al.*, 2003 J Biol Chem 278: 38194-38205). For quality control, single clones were randomly picked and sequenced.

[00411] For the selection of affinity improved binders phage derived from maturation libraries were subjected to three rounds of solution panning using biotinylated human FGFR2. Stringency was increased by lowering the antigen concentration in each panning round (Low *et al.*, 1996 J Mol Biol 260(3): 359-368). In addition to antigen reduction, off-rate selection (Hawkins *et al.*, 1992 J Mol Biol 226: 889-896) was performed. This was combined with prolonged washing steps at RT. The general panning procedure was performed as described above (solution panning).

[00412] For selected subcodes, the 2nd round panning output of a conducted H-CDR2 affinity maturation panning was diversified additionally in the L-CDR3. Library generation was exactly done as described above. For the selection of improved binders, the new generated libraries were subjected into two additional rounds of solution panning.

[00413] The sequence unique clones that were identified were expressed as IgGs and affinity to human FGFR2 was determined using either SET or Biacore[®] methodologies similar to those described in examples 1 and 2. More than 80 clones were evaluated from the 3 parental antibodies and affinity improvements of 3-6 fold were obtained for clones derived from the 12433 antibody, 27-fold for 10164 derived clones and 68-fold for 12425 derived antibodies. The highest affinity clone derived from 10164 was designated 20809 and it's affinity for human FGFR2 was determined to be 450 pM. Interestingly, this antibody also bound to human FGFR4 with an affinity estimate of 7.4 nM. The highest affinity antibody derived from 12425 was designated

20811 (Table 1) and its affinity for human FGFR2 was determined to be 110 pM. The affinity of this antibody to FGFR4 was determined to be 75 pM.

[00414] In additional studies, both 20809 and 20811 were directly conjugated to SMCC-DM1 to yield 20809-MCC-DM1 and 20811-MCC-DM1. The ability of these ADCs to inhibit the growth of SNU16 cells is shown in FIG. 18 (A). The impact of affinity on *in vivo* antitumor activity was assessed in the FGFR2 amplified SNU16 xenograft model. Female nude mice were implanted subcutaneously with 10×10^6 cells in a suspension containing 50% phenol red-free matrigelTM (BD Biosciences) in Hank's balanced salt solution. The total injection volume containing cells in suspension was 200 μ l. Mice were enrolled in the study seven days post implantation with an average tumor volume of 217.6 mm³. After being randomly assigned to one of five groups (n = 6/group), mice received PBS (10 ml/kg) or a 3 mg/kg i.v. dose of 12425-MCC-DM1, 20811-MCC-DM1, 10164-MCC-DM1, or 20809-MCC-DM1. Tumors were calipered twice per week (FIG. 18 (B)). 10164-MCC-DM1 and its affinity matured variant 20809-MCC-DM1 demonstrated similar activity against the SNU16 xenograft. 12425-MCC-DM1 demonstrated superiority over its affinity matured variant 20811-MCC-DM1 with regards to antitumor activity. These data suggest that affinity maturation did not yield improved activity *in vivo* as MCC-DM1 ADCs.

Example 22: Epitope mapping of FGFR2 and its antibody complex by deuterium exchange mass spectrometry (HDx-MS)

[00415] Deuterium exchange mass spectrometry (HDx-MS) measures the deuterium uptake on the amide backbone of a protein. These measurements are sensitive to the amide's solvent accessibility and to changes in the hydrogen bonding network of the backbone amides. HDx-MS is often used to compare proteins in two different states, such as apo and ligand-bound, and coupled with rapid digestion with pepsin. In such experiments one can locate regions, typically of 10 to 15 amino acids, that show differential deuterium uptake between two different states. Regions that are protected are either directly involved in ligand binding or allosterically affected by binding of the ligand.

[00416] In these experiments, the deuterium uptake of *E. Coli.* derived FGFR2 D2-D3 protein (SEQ ID NO:135, see below) was measured in the absence and presence of three therapeutic antibodies: 12425, 10164, and 12433. Regions in FGFR2 that show a decrease in deuterium uptake upon binding of the antibody are likely to be involved in the epitope; however,

due to the nature of the measurement it is also possible to detect changes remote from the direct binding site (allosteric effects). Usually, the regions that have the greatest amount of protection are involved in direct binding although this may not always be the case. In order to delineate direct binding events from allosteric effects orthogonal measurements (*e. g.* X-ray crystallography, alanine mutagenesis) are required.

Table 20. FGFR2 D2-D3 Construct

SEQ ID NO: 135
LENGTH: 237 amino acids
TYPE: Protein
ORGANISM: Human
MAEDFVSENSNNKRAPYWTNTEKMEKRLHAVPAANTVKFRCPAGGNPMPTMRWLKNG KEFKQEHRIGGYKVRNQHWSLIMESVVP SDKGNYTCVVENEYGSINHTYHLDVVERS PH RPILQAGLPANASTVVG DVEFVCKVYSDAQPHIQWIKHVEKNGSKYGPDGLPYLKVLK HSGINSSNAEVLALFNVTEADAGEYICKVSNYIGQANQSAWLT VLPKQQAPGREKEHH HHH

[00417] The epitope mapping experiments described in this example were performed on a Waters HDx-MS platform, which includes a LEAP autosampler, nanoACQUITY UPLC System, and Synapt G2 mass spectrometer. The studies were automated by a LEAP autosampler operated by the LeapShell software, which performed initiation of the deuterium exchange reaction, reaction time control, quench reaction, injection onto the UPLC system and digestion time control. The Leap autosampler was equipped with two temperature controlled stacks maintained at 25 °C for HDx reaction and maintained at 2 °C for storage of protein and quench solution, respectively. Triplicate control experiments were carried out on antigen at a deuterium exchange time of 25 minutes. HDX was quenched with quenching buffer (6 M urea and 1 M TCEP pH = 2.5). After quenching, the antigen was injected into the UPLC system where it is subjected to on-line pepsin digestion at 12 °C followed by a rapid 8 min 2 to 35% acetonitrile gradient over a Waters BEH C18 1 x 100 mm column (maintained at 1 °C) at a flow rate of 40 µL/min. Triplicate experiments were carried out on antigen-mAb complex just as the control experiments except in these experiments the antigen in incubated for 30 min with the antibody at 25 °C prior to deuterium exchange.

[00418] The results of these measurements are summarized in FIG. 19 (A) and FIG. 19 (B). In FIG. 19 (A) the average deuterium uptake for FGFR2 peptides in the absence (control) and presence of mAbs is indicated. In this Figure it is useful to examine two differences: differences between control and mAbs and differences among mAb groups. In FIG. 19 (B), the difference in the deuterium uptake between the mAb and control samples is divided by the standard error in the measurement. Differences are considered significant if they are greater than 0.5 Da in absolute scale (FIG. 19 (A)) and if the ratio of the difference divided by the standard error is less than or equal to -3.0 (FIG. 19 (B)). From this analysis we can rank order regions of the FGFR2 that are protected upon mAb binding into categories of high, medium, and low amounts of protection. FGFR2 regions of high protection are potentially involved in the formation of the epitope with a specific antibody although one cannot rule out other contributions for lesser protected regions.

[00419] Upon binding of 10164 to FGFR2 we observe high amounts of protection in the following two regions of FGFR2: residues 174-189 and 198-216 of SEQ ID NO: 137. These regions are located in the D2 domain specifically consisting of β B to N-terminal side of β C and β C' loop to the β E (FIG. 19 (C) and (D)). There is one region of low protection found in the regions 222-231. However, this region is protected upon binding of all studied antibodies; this observation suggests that this region undergoes a stabilization of its local hydrogen bonding network that may be non-specific and allosteric in nature. Binding of the antibody 12425 to FGFR2 also cause a high amount of protection in the regions of residues 174-189 and 198-216 of SEQ ID NO: 137. In addition, with 12425 family a high amount of protection in the region of residues 160-173 of SEQ ID NO: 137 (FIG. 19 (A) and (B)) was also observed. These regions comprises a region of FGFR2 that is known to interact with FGF2 and FGF1 from published crystallography data (Plotnikov *et al.*, *M. Cell* 2000, 101, 413-424; Beenken *et. al.*, *M. J. Biol. Chem.* 2012, 287, 3067-3078). Protection in this region is unique to the 12425 family of antibodies. Of the antibodies studied, 12433 is unique in that it specifically targets the IIIb isoform of FGFR2 through interactions with the D3 domain of FGFR2. For 12433 high amounts of protection (~2 Da) in the peptide 338-354, and insignificant amounts of protection in a shorter N-terminal fragment from 338-345 were observed. This observation suggests that the portion of the 338-354 peptide that is protected upon 12433 binding is the region 346-354 (FIG. 19 (A) and (B)). The region 346 to 354 contains four residues that are specific to IIIb isoform relative to the

IIIc isoform; these residues include: Q348, A349, N350, and Q351. In additional studies, affinity matured versions of 10164 and 12425 (see Example 23 below) were evaluated and were found to be similar to their respective parental antibodies in terms of the protected regions observed.

[00420] Additionally, using similar methods deuterium exchange studies examining the protection of FGFR4 in the presence of FGFR2/4 cross-reactive antibody 12425 were performed. Preliminary analysis using an FGFR4 D1-D3 construct (SEQ ID NO:124, see below) suggested that binding of 12425 does not cause any protection in the D3 region. Protection appears to be limited to regions within the D1 and D2 domains.

Table 21. FGFR4 D1-D3 Construct

SEQ ID NO: 124
LENGTH: 371 amino acids
TYPE: Protein
ORGANISM: Human
LEASEEVELEPCLAPSLEQQEQELTVALGQPVRLCCGRAERGHHWYKEGSRLAPAGRVR GWRGRLEIASFLPEDAGRYLCLARGSMIVLQNLTLITGDSLTSNDDDEPKSHRDPSNRHS YPQQAPYWTHPQRMEKKLHAVPAGNTVKFRCPAAGNPTPTIRWLKDGQAFHGENRIGGI RLRHQHWSLVMESVVPDRGTYTCLVENAVGSIRYNYLLDVLERSPHRPILQAGLPANTT AVVGSDVELLCKVYSDAQPHIQWLKHIVINGSSFGADGFPYVQVLKTADINSSEVEVLYL RNVSAEDAGEYTCLAGNSIGLSYQSAWLTVLPEEDPTWTAAPEAR YTDKLEFRHDSGL NDIFEAQKIEWHE

Example 23: X-ray crystallographic structure determination of the human FGFR2 / 12425 Fab and FGFR4 / 12425 Fab complexes

[00421] The crystal structure of a human FGFR2 ECD fragment (FGFR2 domain 2, or FGFR2 D2, SEQ ID NO: 138, Table 22) or a human FGFR4 ECD fragment (FGFR4 domain 2, or FGFR4 D2, SEQ ID NO: 143, Table 22) bound to Fab fragment of 12425 (Table 22) are determined. As detailed below, FGFR2 D2 or FGFR4 D2 were expressed, purified and mixed with 12425 Fab to form a complex. Protein crystallography was then employed to generate atomic resolution data for FGFR2 D2 or FGFR4 D2 bound to 12425 Fab to define the epitopes. The crystallographic epitope of FGFR2 was further validated by mutating critical epitope residues and measuring the binding of the mutant FGFR2 to 12425 with surface plasmon resonance (SPR) technology.

Protein production for crystallography and SPR

[00422] The sequences of the proteins produced for crystallography and SPR are shown in Table 22. The construct of FGFR2 D2 comprises residues 146 to 249 (underlined) of human FGFR2 (UniProt identifier P21802-3, SEQ ID NO: 137), along with N- and C-terminal residues from the recombinant expression vector (shown in lower case letters, SEQ ID NO: 137). The construct of FGFR4 D2 (SEQ ID NO: 143) comprises residues 140 to 242 (underlined) of human FGFR4 (UniProt identifier P22455-1, SEQ ID NO: 142), along with N- and C-terminal residues from recombinant expression vector (shown in lower case letters). The construct of FGFR2 domain 2 to domain 3 (D2D3) comprises residues 140 to 369 (Italic letters in SEQ ID NO 137). Both wildtype (WT) and K176A/R201A double mutant versions of FGFR2 D2D3 are produced for epitope validation by SPR (SEQ ID NO: 144 and 145, respectively; lower case letters are N- and C-terminal residues from recombinant expression vector). For 12425 Fab, the sequences of heavy and light chains are shown (SEQ ID NO: 139 and 140, respectively).

Table 22: Sequences of proteins used for crystallography and SPR

Construct	Amino acid sequence in one letter code	SEQ ID NO
Human FGFR2 (P21802-3)	MVSWGRFICLVVVTMATLSLARPSFSLVEDTTLEPEEPPTKYQ ISQPEVYVAAPGESLEVRCLLKDAAVISWTKDGVHLGPNNRT VLIGEYLQIKGATPRDSGLYACTASRTVDSETWYFMVNVTD ISSGDDDDTDGAEDFVSE <u>NSNNKRAPYWTNTEKMEKRLHAVP</u> <u>AANTVKFRCPAGGNPMPTMRWLKNGKEFKQEHRIGGYKVRNQ</u> <u>HWSLIMESVVP SDKGNYTCVVENEYGSINHTYHLDVVERS</u> <u>PHRPI</u> LQAGLPANASTVVGGDVEFVCKVYSDAQPHIQWIKHVEKNGSKY GPDGLPYLKVLKHSGINSSNAEVLALFNVT EADAGEYICKVSNYIG QANQSAWLT VLPKQQAPGREKEITASPDYLEIAIYCIGVFLIAC MVVTVILCRMKN TTKKPDFSSQPAVHKLTKRIPLRRQVTVSA ESSSSMNSNTPLVRITTRLSS TADTPMLAGVSEYELPEDPKWE FPRDKLTLGKPLGEGCFGQVVM AEAVGIDKDKPKEAVTVAV KMLKDDATEKDLS DLVSEMEMMMKMIGKHKNINLLGACTQD GPLYVIVEYASKGNLREYLRARRPPGMEYSYDINRVPEEQMT FKDLVSCTYQLARGMEY LASQKCIHRDLAARNVLVTENNVM KIADFGLARDINNIDYYKKTTNGRLPVK WMAPEALFDRVYT	137

	HQSDVWSFGVLMWEIFTLGGSPYPGIPVEELFKLLKEGHRMD KPANCTNELYMMMRDCWHAVPSQRPTFKQLVEDLDRILTLT TNEEYLDLSQPLEQYSPSYPDTRSSCSSGDDSVFSPDPMPYEP CLPQYPHINGSVKT	
FGFR2 D2	mNSNNKRAPYWTNTEKMEKRLHAVPAANTVKFRCPAGGNP MPTMRWLKNGKEFKQEHRIGGYKVRNQHWSLIMESVVP KGNVTCVVENEYGSINHTYHLDVVlvprgslhshhshh	138
12425 Fab heavy chain	QVQLLESGGGLVQPGGSLRLSCAASGFTFSDYAMSWVRQAP GKGLEWVSVIEGDGSYTHYADSVKGRFTISRDN SKNTLYLQ MNSLRAEDTAVYYCAREKTYSSAFDYWGQGTLVTVSSASTK GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKRVEPKSCDKTH	139
12425 Fab light chain	DIQMTQSPSSLSASVGDRVITITCRASQDISSDLN WYQQKPGK APKLLIYDASN LQSGVPSRFSGSGSGTDFTLTISSLQPEDFAVY YCQQHYSPSHTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKD STYSLSSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRG EC	140
Human FGFR4 (P22455-1)	MRLLLALLGVLLSVPGPPVLSLEASEEVELEPCLAPSLEQQEQ ELTVALGQPVRLCCGRAERGGHWYKEGSRLAPAGRVRGWR GRLEIASFLPEDAGRYLCLARGSMIVLQNLTLITGDSLTSND DEDPKSHRDPSNRHSYPQQAPYWTHPQRM EKKLHAVPAGNT <u>VKFRCPAAGNPTPTIRWLKDQGAFHGENRIGGIRLRHQHWSL</u> <u>VMESVVP</u> SDRGTYTCLVENAVGSIRYNYLLDV LERSPHRPIL QAGLPANTTAVVGSDVELLCKVYSDAQPHIQWLKHIVINGSS FGADGFPYVQVLKTADINSSEVEVLYLRNVSAEDAGEYTCLA GNSIGLSYQSAWLTVLPEEDPTWTAAAP EARYTDIILYASGSL ALAVLLLLAGLYRGQALHGRHPRPPATVQKLSRFPLARQFSL ESGSSGKSSSSLVRGVRLSSSGPALLAGLVSLDLPLDPLWEFP RDRLVLGKPLGEGCFGQVVRAEAFGMDPARPDQASTVAVK MLKDNASDKDLADLVSEMEVMKLIGRHKN IINLLGVCTQEG PLYVIVECAAKGNLREFLRARRPPGPD LSPDGPRSSSEGPLSFPV LVSCAYQVARGMQYLESRKCIHRDLAARNVLVTE DNVMKIA	142

	DFGLARGVHHIDYYKKTSNGRLPVKWMAPEALFDRVYTHQS DVWSFGILLWEIFTLGGSPYPGIPVEELFSLREGHRMDRPPH CPPELYGLMRECWHAAPSQRPTFKQLVEALDKVLLAVSEY LDLRLTFGPYSPSGGDASSTCSSSDSVFSDPLPLGSSSFPGS GVQT	
FGFR4 D2	mHSYPQQAPYWTHPQRMEKKLHAVPAGNTVKFRCPAAGNP TPTIRWLKDGQAFHGENRIGGIRLRHQHWSLVMESVPSDRG TYTCLVENAVGSIRYNYLLDVLLvprgslehhhhh	143
FGFR2 D2D3	mAEDFVSENSNNKRAPYWTNTEKMEKRLHAVPAANTVKFR CPAGGNPMPTMRWLKNGKEFKQEHRRIGGYKVRNQHWSLIM ESVVPSDKGNYTCVVENEYGSINHTYHLDVVERSHPRPILQA GLPANASTVVGGDVEFVCKVYSDAQPHIQWIKHVEKNGSKY GPDGLPYLKVLKHSGINSSNAEVLALFNVTEADAGEYICKVS NYIGQANQSAWLTVLPKQQAPGREKEhhhhh	144
FGFR2 D2D3 K176A/R210A	mAEDFVSENSNNKRAPYWTNTEKMEKRLHAVPAANTV <u>A</u> FR CPAGGNPMPTMRWLKNGKEFKQEHRRIGGYKV <u>A</u> NQHWSLIM ESVVPSDKGNYTCVVENEYGSINHTYHLDVVERSHPRPILQA GLPANASTVVGGDVEFVCKVYSDAQPHIQWIKHVEKNGSKY GPDGLPYLKVLKHSGINSSNAEVLALFNVTEADAGEYICKVS NYIGQANQSAWLTVLPKQQAPGREKEhhhhh	145

[00423] FGFR2 D2 was expressed in *E.coli* BL21 (DE3) (Novagen^R, EMD Millipore). Following overnight induction with IPTG at 18 °C, cells were harvested, frozen at -80 °C and lysed. The inclusion body was extracted with 20 mM sodium phosphate pH 7.5, 300 mM NaCl and 8M urea, and loaded onto a Ni-NTA column pre-equilibrated in the same buffer. The column was washed with 20 mM sodium phosphate pH 7.5, 300 mM NaCl, 50 mM ammonium sulfate and 20mM imidzole, followed by elution with 20 mM sodium phosphate pH 7.5, 300mM NaCl, 50mM ammonium sulfate and 300mM imidazole. The eluted protein was then diluted 12-fold with 20 mM sodium phosphate pH 6.5 (buffer A), and loaded onto HiTrap S HP column (GE Healthcare), pre-equilibrated in buffer A plus 2% of 20mM sodium phosphate pH 6.5, 1.5M NaCl, 50mM ammonium sulfate (buffer B). The S column was eluted by a gradient of buffer A plus 2% - 100% buffer B. The major peak containing FGFR2 D2 was collected, concentrated, and loaded onto HiLoad® 16/60 SuperdexTM 75 (GE Healthcare) equilibrated in 20mM Hepes pH 7.5,

150mM NaCl. Peak fractions were analyzed by SDS-PAGE and LCMS and then pooled to form a complex with 12425 Fab.

[00424] FGFR4 D2 was expressed in *E.coli* strain Shuffle® T7 Express (New England BioLabs®). Following overnight induction with IPTG at 18 °C, cells were harvested and lysed in 50 mM sodium phosphate pH 7.5, 300 mM NaCl plus 0.1 mg/ml deoxyribonuclease I from bovine pancreas (Sigma®) and 1 tablet of cOmplete Protease Inhibitor Cocktail Tablets (Roche®) per 50ml of lysis buffer. The lysis was clarified by centrifugation at 15,000 rpm for 1hr; Talon^R resin (Clontech®) was then added to the supernatant for batch binding overnight at 4 °C. The next day the resin was packed into a column, washed with 50 mM sodium phosphate pH 7.5, 300 mM NaCl, 20 mM imidazole, and eluted with 50 mM sodium phosphate pH 7.5, 300 mM NaCl, 300 mM imidazole. The eluted protein was then diluted 12 fold with 50mM sodium phosphate pH 7.5 (buffer C), and loaded onto HiTrap S HP column (GE Healthcare) pre-equilibrated in buffer C plus 2% of 50 mM sodium phosphate pH 7.5, 1M NaCl (buffer D). The S column was eluted by a gradient of buffer C plus 2% - 100% buffer D. The major peak containing FGFR4 D2 was collected, concentrated, and loaded onto HiLoad® 16/60 SuperdexTM 75 (GE Healthcare) equilibrated in 20mM Hepes pH 7.5, 150mM NaCl. Peak fractions were analyzed by SDS-PAGE and LCMS, then pooled to form a complex with 12425 Fab.

[00425] 12425 Fab is produced by cleavage of full-length 12425 IgG with immobilized papain (Pierce). 12425 IgG at 20 mg/ml in 20 mM sodium phosphate pH 7.0 and 10 mM EDTA was mixed with immobilized papain at a weight ratio of 80:1. The mixture was rotated in a 15 ml tube at 37 °C overnight. The next day the immobilized papain was removed by gravity flow column; the flow through, which contains both Fab and Fc segments, was collected and loaded onto HiTrapTM MabSelect SURETM column (GE Healthcare) to remove the Fc segment. The flow through from this step, which contains only the Fab fragment, was concentrated and loaded onto HiLoad® 16/60 SuperdexTM 75 (GE Healthcare) equilibrated in 20mM Hepes pH 7.5, 150mM NaCl. Peak fractions were analyzed by SDS-PAGE and LCMS, and then pooled to form a complex with FGFR2 D2 or FGFR4 D2.

Crystallization and structure determination of FGFR2 D2/12425 and FGFR4 D2/12425 Fab complexes

[00426] The complex of FGFR2 D2 or FGFR4 D2 with 12425 Fab was prepared by mixing the purified FGFR2 D2 or FGFR4 D2 with 12425 Fab at a 2:1 molar ratio (concentration

measured via LCUV), incubating on ice for 30 min, and purifying the complex on a HiLoad® 16/60 Superdex™ 75 (GE Healthcare) equilibrated in 20mM Hepes pH 7.5, 150mM NaCl. Peak fractions were analyzed by SDS-PAGE and LCMS.

[00427] To crystalize the FGFR2 D2/12425 Fab complex, fractions containing the complex were pooled and concentrated to about 30 mg/ml. Trypsin (dissolved in 1 mM HCl and 2 mM CaCl₂ at 1 mg/ml) was added into the complex at a volume ratio of 1:100 to facilitate crystallization (Wernimont *et al.*, (2009) Plos One 4:e5095). The FGFR2 D2/12425 Fab/trypsin mixture was immediately centrifuged and screened for crystallization.

[00428] Crystals were grown by sitting drop vapor diffusion. In detail, 0.1 µl of protein was mixed with 0.1 µl of reservoir solution which contains 0.1 M tri-sodium citrate dihydrate pH 5.0, 20% (w/v) PEG6000; and the drop was equilibrated against 45 µl of the same reservoir solution at 4 °C.

[00429] To crystalize FGFR4 D2/12425 Fab complex, size exclusion fractions containing the complex were pooled, concentrated to 20mg/ml, centrifuged and screened for crystallization. Crystals were grown in a same way as the FGFR2 D2/12425 Fab complex (described above), except that the reservoir solution contains 0.2 M magnesium formate, 20% (w/v) PEG3350 and the crystal were grown at 20 °C.

[00430] Before data collection, both the FGFR2 D2/12425 Fab and FGFR4 D2/12425 Fab crystals were transferred to a reservoir solution containing an additional 22.5% glycerol and flash cooled in liquid nitrogen.

Diffraction data were collected at beamline 17-ID at the Advanced Photon Source (Argonne National Laboratory, USA). Data were processed and scaled at 2.8Å using HKL2000 (HKL Research). Both structures were solved by molecular replacement using Phaser (McCoy *et al.*, (2007) J. Appl. Cryst. 40:658-674) with FGFR2 D2 structure (PDB ID: 3DAR) and an in-house Fab structure as search models. The final models were built in COOT (Emsley & Cowtan (2004) Acta Cryst. 60:2126-2132) and refined with Buster (Global Phasing, LTD). Residues of FGFR2 D2 or FGFR4 D2 that contain atoms within 5 Å of any atom in the 12425 Fab are identified by PyMOL (Schrödinger, LLC) and listed in Tables 23 and 24.

Mutagenesis of FGFR2 and SPR assay

[00431] K176A/R210A double mutation was introduced into FGFR2 D2D3 construct by Quikchange^R Site-Directed Mutagenesis kit (Agilent Technologies). Both WT and mutant FGFR2 D2D3 proteins were produced using the same procedure as for FGFR4 D2 protein.

[00432] For the reagents used in the SPR assay, affinity pure F(ab)' fragment anti-human IgG Fc γ fragment (109-006-098) was purchased from Jackson Immuno Research Laboratory. Sensor chip CM5 (BR-1006-068), Glycine pH 2.0 (BR-1003-55) and HBS-EP⁺ buffer (BR-1006-69) were ordered from GE Healthcare. BSA is from Gibcor[®] (15260) and Heparin from Sigma^R (H3149-100KU).

[00433] All the SPR work was performed using Biacore[®] T100 with evaluation software version 1.1 and CM5 sensor chip. HBS-EP⁺ with 0.5 mg/ml of BSA and 10 μ g/ml Heparin was used as running buffer for all experiments. The immobilization level and analyte interaction were measured by response unit (RU). Pilot experiments were performed to test and confirm the feasibility of the immobilization of anti-human Fc antibody and subsequent capture of 12425 IgG.

[00434] For 12425 kinetics measurement, antibody was captured on the chip, and then WT or mutant FGFR2 D2D3 protein was flowed over. Briefly, 50 μ g/ml of anti-human Fc antibody at pH 4.75 was immobilized on CM5 chip through amine coupling at a flow rate of 10 μ l/minute on all four flow cells to reach 6000 RU. 3 μ g/ml of 12425 was injected at 10 μ l/minute for 10 seconds over test flow cell. 0.078 -100 nM of FGFR2 D2D3 protein at 2 fold dilution was injected at 80 μ l/minute for 7 minutes over all reference flow cell and test flow cell. HBS-EP⁺ + BSA + Heparin buffer was then flowed for 10 minutes to dissociate bound 12425. After each injection cycle, the chip surface was regenerated with 10 mM glycine pH 2 at 60 μ l/minute for 70 seconds. All kinetics measurements were done at 25°C.

FGFR2 epitope for 12425

[00435] The crystal structure of the FGFR2 D2/12425 Fab complex was used to identify the FGFR2 epitope for 12425. There are six copies of the FGFR2 D2/12425 Fab complex in the asymmetric unit of the crystal (an asymmetric unit contains all the structural information needed to reproduce the whole crystal). All six copies share almost identical residues in contact with 12425 Fab except for small variations due to crystal packing. Only those 12425-contacting FGFR2 residues that are shared by all six copies were used to define epitope.

[00436] The interaction surface on FGFR2 D2 by 12425 Fab is formed by several discontinuous (*i.e.*, noncontiguous) sequences: namely residues 173 through 176, residue 178, residues 208 through 210, and residues 212, 213, 217 and 219, as detailed in Table 23 and 24. These residues form the three-dimensional surface that is bound by 12425 Fab (FIG. 20 (A)). This epitope defined by crystallography is in good agreement with that defined by deuterium exchange mass spectrometry (HDx-MS), which comprises residues 160-173, 174-189 and 198-216.

Table 23. Interactions between human FGFR2 D2 and 12425 Fab heavy chain (H). FGFR2 residues are numbered based upon P21802-3 (SEQ ID NO: 137). Fab heavy chain residues are numbered based upon their linear amino acid sequence (SEQ ID NO: 139). FGFR2 residues shown have at least one atom within 5 Å of an atom in the 12425 Fab, to account for potential water mediated interactions.

Human FGFR2		12425 Fab		
Amino acid	Number	Amino acid	Number	Chain
LYS	208	HIS	59	H
VAL	209	TYR	57	H
ARG	210	ALA	33	H
		VAL	50	H
		TYR	57	H
		GLU	52	H
		GLU	99	H
		SER	104	H
GLN	212	GLU	52	H
		ASP	54	H
HIS	213	GLU	52	H
		SER	104	H

Table 24. Interactions between human FGFR D2 and 12425 Fab light chain (L). FGFR2 residues are numbered based upon P21802-3 (SEQ ID NO: 137). Fab light chain residues are numbered based upon their linear amino acid sequence (SEQ ID NO: 140). FGFR2 residues shown have at least one atom within 5 Å of an atom in the 12425 Fab, to account for potential water mediated interactions.

Human FGFR2		12425 Fab		
Residue	Number	Residue	Number	Chain
ASN	173	GLN	27	L
THR	174	GLN	27	L
		TYR	92	L
VAL	175	TYR	92	L
LYS	176	ASP	32	L
		HIS	91	L
		TYR	92	L
		ASP	32	L
ARG	178	ASP	32	L
ARG	210	HIS	96	L
ILE	217	TYR	92	L
		PRO	94	L
GLU	219	SER	93	L
		PRO	94	L

[00437] From the FGFR2 D2/12425 Fab structure, the potential N-linked glycosylation sites on FGFR2, namely Asn241 and Asn288 (Duchesne *et al.*, (2006) J. Biol. Chem 281:27178-27189) are on the opposite surface of the protein away from the 12425 epitope (FIG. 20 (A)), indicating that 12425 binding to FGFR2 is independent of glycosylation. This is consistent with the finding that 12425 antibody binds *E.coli*-produced (no glycosylation) and mammalian cell-produced (glycosylated) FGFR2 D2-D3 with similar affinity (data not shown).

[00438] Lys176 and Arg210 of FGFR2 D2 are the two epitope residues making most contacts with 12425 Fab light and heavy chains, respectively. Interestingly, Arg210 has been demonstrated to bind heparin (Pellegrini *et al.*, (2000) Nature 407:1029-1034), which can enhance FGFR2 binding to FGFs and subsequent dimerization and signaling. Meanwhile, Lys176 is also postulated to be in the heparin binding pocket (Pellegrini *et al.*, (2000) Nature 407:1029-1034). Mutation of these two residues to alanine completely abolishes binding of FGFR2 to 12425 IgG in SPR assays (see above for SPR methods), which confirmed their critical role in binding 12425 and validated the epitope observed in the crystal structure.

FGFR4 epitope for 12425

[00439] The crystal structure of the FGFR4 D2/12425 Fab complex was used to identify the FGFR4 epitope for 12425. There were four copies of FGFR4 D2/12425 Fab complex in the asymmetric unit of the crystal. All four copies share almost identical residues in contact with 12425 Fab except for small variations due to crystal packing. Only those 12425-contacting FGFR4 residues that are shared by all four copies were used to define the epitope.

[00440] The interaction surface on FGFR4 D2 by 12425 Fab was formed by several discontinuous (*i.e.*, noncontiguous) sequences: namely residues 150-151, 154, 157, 160, 166-169, 171, 173-174, 201-207, 210 and 212, as detailed in Table 25. These residues form the three-dimensional surface that is bound by 12425 Fab (FIG. 20 (B)).

Table 25. Interactions between human FGFR4 D2 and 12425 Fab heavy (H) and light (L) chain. FGFR4 residues are numbered based upon P22455-1 (SEQ ID NO: 142). Fab residues are numbered based upon their linear amino acid sequence (SEQ ID NO: 139 and 140). FGFR4 residues shown have at least one atom within 5 Å of an atom in the 12425 Fab, to account for potential water mediated interactions.

Human FGFR4		12425		
Amino acid	Number	Amino acid	Number	Chain
THR	150	TYR	102	H
HIS	151	TYR	102	H
PRO	173	TYR	102	H
ALA	174	TYR	102	H
ARG	201	TYR	57	H
		THR	58	H
		HIS	59	H
LEU	202	TYR	57	H
ARG	203	ALA	33	H
		VAL	50	H
		GLU	52	H
		TYR	57	H
		GLU	99	H
		SER	104	H
HIS	204	ASP	54	H

		SER	56	H
GLN	205	GLU	52	H
		GLY	53	H
		ASP	54	H
		SER	56	H
		TYR	57	H
		THR	101	H
		TYR	102	H
HIS	206	GLU	52	H
		GLU	99	H
		LYS	100	H
		THR	101	H
		TYR	102	H
		SER	103	H
		SER	104	H
TRP	207	TYR	102	H
GLU	212	HIS	59	H
ARG	154	SER	30	L
		SER	31	L
		ASP	32	L
		ASP	50	L
LYS	157	ASP	28	L
HIS	160	TYR	92	L
ASN	166	GLN	27	L
THR	167	ILE	2	L
		GLN	27	L
		ASP	28	L
		TYR	92	L
		SER	93	L
		PRO	94	L
VAL	168	TYR	92	L

LYS	169	ASP	32	L
		HIS	91	L
		TYR	92	L
ARG	171	SER	30	L
		ASP	32	L
		HIS	91	L
		TYR	92	L
ARG	201	PRO	94	L
ARG	203	HIS	96	L
VAL	210	TYR	92	L
		PRO	94	L
GLU	212	SER	93	L
		PRO	94	L
		SER	95	L

Comparison of 12425 epitopes on FGFR2 and FGFR4

[00441] The 12425 epitopes on FGFR2 and FGFR4 are very similar in both sequence and conformation. As shown in the sequence alignment (FIG. 20 (C)), all epitope residues (in dashed boxes) on FGFR2 are shared by and conserved in FGFR4. Furthermore, when the two complex structures are superposed on the variable domains of 12425, the structure of the two antigens overlay very well, indicating the conformations of 12425 binding to FGFR2 and FGFR4 are very similar. This provides structural explanation for the cross-reactivity of 12425 to both receptors.

12425 binding and FGFR dimerization

[00442] Two models have been reported for how FGFR-FGF-heparin complex dimerizes on the cell surface to activate downstream signaling, namely the 2:2:2 model (FGFR:FGF:heparin)(Schlessinger *et al.*, (2000) Mol. Cell 6:743-750), and the 2:2:1 model (FGFR:FGF:heparin)(Pellegrini *et al.*, (2000) Nature 407:1029-1034). Based on the crystal structure of FGFR2 D2/12425 Fab complex, the binding of 12425 to FGFR2 and/or FGFR4 clashes with and thus could block both dimerization models.

Example 24. Formulation

[00443] The clinical service form (CSF) of the ADC is a lyophilisate in vial containing 50 mg 12425-MCC-DM1, 16.2 mg sodium succinate, 410.8 mg sucrose and 1 mg polysorbate 20

(without considering the overfill of 10 % to allow for withdrawal of the declared content). After reconstitution of the lyophilizate with 5 mL water for injection, a solution containing 10 mg/mL 12425-MCC-DM1, 20 mM sodium succinate, 240 mM Sucrose and 0.02 % polysorbate 20 at a pH of 5.0 is obtained.

[00444] For subsequent intravenous administration, the obtained solution will usually be further diluted into a carrier solution to the ready-to-use ADC solution for infusion.

[00445] For the CSF, an ADC concentration of 10 mg/mL was chosen based on preliminary stability testing. A sucrose concentration of 240 mM was selected in order to create an isotonic formulation, to maintain an amorphous lyophilizate cake structure and to afford protein stabilization.

[00446] Important stability-indicating analytical methods to select the most stable formulation encompassed, amongst others, size-exclusion chromatography to determine aggregation levels, subvisible particulate matter testing, free Toxin determination and potency testing.

[00447] The pre-screening study showed that polysorbate 20 at a concentration of 0.02 % provides sufficient stabilization against mechanical stress. The liquid and lyophilized stability studies at real-time and accelerated stability conditions (25°C and 40°C) demonstrated that a succinate pH 5.0 formulation provides the overall best storage stability. Most notably in this formulation the best balance of all tested formulations between aggregation and release of the free Toxin could be met. After three months at 40°C no noteworthy increase in degradation products could be determined.

Example 25. Tumor resistance mechanisms and the use of combination therapies

[00448] Preexisting heterogeneity is a common feature of numerous cancers. Clinically significant tumors (and the cell lines derived from them) are many generations away from their initial oncogenic event. They have had ample time to acquire genetically diverse sub-populations (Nowell, Science 194: 23-28 (1976)) that will not necessarily be dependent on a common genetic driver lesion for survival. If these sub-populations are fit enough to be carried along in successive generations, rather than treating a single clonal tumor in each patient, we are, in fact, treating a complex population. Thus, the existence of clonal populations with different amplification of oncogenes or tumor suppressors could explain sensitivity or resistance to single therapy and require targeted combination therapies.

FGFR2 Expression is heterogeneous in FGFR2 amplified SNU 16 Cells and in FGFR2 amplified human tumors

[00449] FGFR2 expression was measured in SNU16 cells by immunohistochemistry (IHC), flow cytometry and/or fluorescence in situ hybridization using methods known in the art and described herein. IHC staining of parental SNU 16 paraffin embedded cell pellets revealed a range of FGFR2 staining intensities. In addition, FGFR2 surface expression on SNU 16 cells propagated in culture also showed a range of expression values for FGFR2 as measured by flow cytometry. Interestingly, when the cell population was co-stained for EGFR, there appeared to be a sub-population of EGFR positive cells. Scattered EGFR amplified cells (<5%) were also apparent by FISH in a pretreatment cell block preparation, although when the population was examined by qPCR, no EGFR amplification could be detected in the population prior to treatment, likely due to the low level of expression.

[00450] 296 human gastric tumor samples that were evaluated by qPCR and 7 (2.4%) were found to have an increase in FGFR2 copy number (for example, a gene copy number of approximately 4). IHC staining on 9 samples from a set of those with the highest FGFR2 copy numbers revealed each sample had tumor areas that were positive and areas that were negative for FGFR2 expression. Immunoreactivity did not correlate with any specific tumor morphology. When tumor samples were micro-dissected using the FGFR2 immunoreactivity as a mask on serial sections, DNA from these FGFR2 positive and negative regions correlated with FGFR2 gene amplification or non-amplification, respectively, in all nine cases. The correlation of overexpression and amplification of FGFR2 was confirmed by deep sequencing, as described below.

EGFR is overexpressed and amplified in SNU 16 cells resistant to BGJ398, and FGFR2 expression and amplification is lost

[00451] SNU 16 cells (ACCT CRL-5974) were cultured in 0.5 μ M of BGJ398 with RPMI-1640 medium + 10% FBS (ATCC catalog # 30-2001), followed by increasing concentrations of BGJ398 from 0.5 μ M to 1.0 μ M to 2.5 μ M to 5.0 μ M. Medium was changed and fresh BGJ398 (5 μ M) was added once per week for 6 weeks. The cells were frozen in recovery cell culture freezing media (GIBCO® #12648-101) and reconstituted in RPMI-1640+10% FBS. Thawed passages of the resistant cell line were subsequently maintained under 1 μ M BGJ398 selection. SNU 16 cells kept in culture under selection with BGJ398 eventually became less sensitive to

growth inhibition with IC₅₀ increasing from 1.7 nM to 2.4 μM, in sensitive to resistant cells, respectively.

[00452] Western Blot analysis of BGJ398 resistant SNU 16 cells showed that the resistant cell population had lost expression of FGFR2 and was no longer signaling through p-FRS2, indicating that FGFR2 signaling pathway was not longer active. The resistant population instead expressed high levels of EGFR and pEGFR indicating the cells were instead signaling through the EGFR pathway. BGJ398 resistant SNU 16 cells also exhibit an increase in EGFR DNA copy number, as measured by qPCR, that correlates with an increase in protein expression compared to pretreated SNU16 cells. The BGJ398 resistant cells continue to grow, but at a slower growth rate than the parental (untreated) cell line, with a doubling time of 125 hours compared to 36 hours for the parental SNU 16 cells.

[00453] The onset of BGJ398 resistance in SNU 16 cells results in an acquired sensitivity to the EGFR inhibitor Gefitinib (FIG 21). Cell Growth assays done on SNU 16 parental and BGJ398 resistant cells showed a shift in Gefitinib IC₅₀ from > 10 μM to 16.7 nM, respectively, confirming a switching of oncogene dependence from FGFR2 to EGFR.

[00454] We have shown that a cell line that is amplified in a transforming oncogene, FGFR2, contains pre-existing cells that are amplified and dependent on a second oncogene, EGFR. When the FGFR2 amplified cell line SNU 16 was placed under pressure by an FGFR kinase inhibitor, one population was eliminated and a sub-population became dominant; we anticipate that when a heterogeneous tumor is placed under selective pressure, such as a targeted therapy, the growth of sub-populations may be favored and lead to clinical resistance.

Deep sequencing reveals the pre-existence of additional oncogenic lesions in human gastric cancer specimens

[00455] Deep sequencing of microdissected regions of paired tumor samples, taken from tumor regions overexpressing FGFR2 and tumor regions without FGFR2 overexpression showed a correlation with FGFR2 amplification. All tumor regions with FGFR2 overexpression exhibited FGFR2 amplification in copy number (e.g.: copy number 6 -16), while tumor regions without FGFR2 overexpression did not exhibit FGFR2 copy number amplification. Consistent with the findings in SNU 16, described above, one tumor exhibited EGFR amplification only in the non-FGFR2 amplified region. The EGFR amplification was co-incident with a kras amplification in this specimen. This observation is consistent with previous reports suggesting kras amplification

is a mechanism of resistance in cancers treated with anti-EGFR therapies (Valtorta, *et al.*, IJC (2013) 133:1259-1266; Misale, *et al.*, Nature (2012) 486:532-538).

[00456] Other tumors exhibited amplification of alternate receptor tyrosine kinases. For example, one tumor pair had Her2 amplified in both the FGFR2 amplified and FGFR2 non-amplified region, while an additional tumor had IGF1R amplification identified only in the non-FGFR2 amplified region. All regions in all tumors appeared to have a mutation in p53. Several additional mutations were identified, but were not consistently found across the sample set. These small pre-existing populations, amplified in initially non-prevalent receptor tyrosine kinases, may be a source of resistant cells when selective pressure, such as a targeted therapy (e.g.: anti-FGFR2 therapy), is applied.

[00457] We have also demonstrated that primary human tumors with FGFR2 amplification contain sub-populations with amplifications of other tyrosine kinases, and that this sub-population amplification sometimes occurs in tumor regions without FGFR2 amplification. Reminiscent of other studies (Snuderl, *et al.* (2011) Cancer Cell 20: 810-817), all tumor regions also contained a common mutation, in this case a mutation in p53, suggesting a common ancestor for the tumor as a whole.

FGFR2 as a resistance mechanism in Her2 expressing tumors

[00458] Amplification of FGFR2 has been suggested as a resistance mechanism in breast cancer cells following treatment with lapatinib (Azuma, *et al.* (2011) Biochem Biophys Res Comm, 407:219-224), and FGFR2 expression was seen to be over expressed in breast cancer patients who had poor response to lapatinib. We have demonstrated by deep sequencing that a human Her2 amplified gastric tumor sample has preexisting subpopulations of FGFR2 amplified cells (Deeds *et al.*, United States and Canadian Academy of Pathology, March 2014: Abstract no: 453290). This suggests that FGFR2 amplification might represent a resistance mechanism in other Her2 amplified tumors following anti-Her2 therapy. Thus, treatment of a Her2 therapy in combination with an FGFR2 therapy, for example the FGFR2 antibodies and ADCs described herein would be useful for treatment of Her2 expressing and/or Her2 resistant cancers.

[00459] These data suggested that FGFR2 therapies, for example the FGFR2 ADCs described herein, may be useful in combination with other tyrosine kinase inhibitors for the treatment of cancer. In addition, the FGFR2 ADCs described herein may be useful for the treatment of patient populations resistant to other cancer therapies, including other tyrosine kinase

inhibitors (e.g.: Her2 inhibitors, Her3 inhibitors, EGFR inhibitors, Met inhibitors, and IGFR inhibitors).

[00460] It is understood that the examples and embodiments described herein are for illustrative purposes only and that various modifications or changes in light thereof will be suggested to persons skilled in the art and are to be included within the spirit and purview of this application and scope of the appended claims.

We Claim:

1. An antibody drug conjugate of the formula



wherein

Ab is an antibody or antigen binding fragment thereof that specifically binds to both human FGFR2 and FGFR4;

L is a linker;

D is a drug moiety;

m is an integer from 1 to 8; and

n is an integer from 1 to 10.

2. The antibody drug conjugate of claim 1, wherein said m is 1.
3. The antibody drug conjugate of claim 1 or 2, wherein said n is 3 or 4.
4. The antibody drug conjugate of any of claims 1-3, wherein said antibody or antigen binding fragment specifically binds to all isoforms of human FGFR2.
5. The antibody drug conjugate of any of claims 1 -4, wherein said antibody or antigen binding fragment specifically binds to an epitope on human FGFR2 comprising amino acid residues 176 (Lys) and 210 (Arg) of SEQ ID NO:137.
6. The antibody drug conjugate of any of claims 1-5, wherein said antibody or antigen binding fragment specifically binds amino acid residues 173 (Asn), 174 (Thr), 175 (Val), 176 (Lys), 178 (Arg), 208 (Lys), 209 (Val), 210 (Arg), 212 (Gln), 213 (His), 217 (Ile), and 219 (Glu) of SEQ ID NO:137.
7. The antibody drug conjugate of any of claims 1-6, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR2 comprising SEQ ID NO:136 or SEQ ID NO:141.
8. The antibody drug conjugate of any of claims 1-6, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR2 consisting of SEQ ID NO:136 or SEQ ID NO:141.

9. The antibody drug conjugate of any of claims 1-8, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR4 comprising amino acid residues 169 (Lys) and 203 (Arg) of SEQ ID NO: 142.
10. The antibody drug conjugate of any of claims 1-9, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR2 comprising amino acid residues 176 (Lys) and 210 (Arg) of SEQ ID NO:137 and an epitope of human FGFR4 comprising amino acid residues 169 (Lys) and 203 (Arg) of SEQ ID NO: 142.
11. The antibody drug conjugate of any of claims 1-10, wherein said antibody or antigen binding fragment specifically binds amino acid residues 150 (Thr), 151 (His), 154 (Arg), 157 (Lys), 160 (His), 166 (Asn), 167 (Thr), 168 (Val), 169 (Lys), 171 (Arg), 173 (Pro), 174 (Ala), 201 (Arg), 202 (Leu), 203 (Arg), 204 (His), 205 (Gln), 206 (His), 207 (Trp), 210 (Val) and 212 (Glu) of human FGFR4 of SEQ ID NO:142.
12. The antibody drug conjugate of any of claims 1-11, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR4 comprising SEQ ID NO: 132 or 133.
13. The antibody drug conjugate of any of claims 1-11, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR4 consisting of SEQ ID NO: 132 or 133.
14. The antibody drug conjugate of any of claims 1-13, wherein said antibody or antigen binding fragment thereof comprises a heavy chain variable region that comprises: (a) a VH CDR1 of SEQ ID NO: 1, 21, 41, 61, 81, or 101, (b) a VH CDR2 of SEQ ID NO: 2, 22, 42, 62, 82 or 102 and (c) a VH CDR3 of SEQ ID NO: 3, 23, 43, 63, 83, or 103 wherein the CDR is defined in accordance with the Kabat definition.
15. The antibody drug conjugate of claim 14, wherein said antibody or antigen binding fragment thereof further comprises a light chain variable region that comprises: (a) a VL CDR1 of SEQ ID NO: 11, 31, 51, 71, 91, or 111, (b) a VL CDR2 of SEQ ID NO: 12, 32, 52, 72, 92, or 112 and a VL CDR3 of SEQ ID NO: 13, 33, 53, 73, 93, or 113 wherein the CDR is defined in accordance with the Kabat definition.
16. The antibody drug conjugate of claim 15, wherein said antibody or antigen binding fragment thereof comprises a VH region of SEQ ID NO: 7, 27, 47, 67, 87, or 107 and a VL region of SEQ ID NO: 17, 37, 57, 77, 97, or 117.

17. The antibody drug conjugate of claim 16, wherein said antibody consists of a heavy chain of SEQ ID NO: 9, 29, 49, 69, 89, or 109 and a light chain of SEQ ID NO: 19, 39, 59, 79, 99, or 119.

18. The antibody drug conjugate of claim 1, wherein said antibody or antigen binding fragment cross competes with an antibody consists of a heavy chain of SEQ ID NO: 9, 29, 49, 69, 89, or 109 and a light chain of SEQ ID NO: 19 for binding to human FGFR2 and FGFR4.

19. The antibody drug conjugate of claim 1, wherein said antibody or antigen binding fragment has enhanced ADCC activity as compared to an antibody consisting of a heavy chain of SEQ ID NO: 9, 29, 49, 69, 89, or 109 and a light chain of SEQ ID NO: 19, 39, 59, 79, 99, or 119.

20. The antibody drug conjugate of claim 1, wherein said antibody or antigen binding fragment does not have enhanced ADCC activity as compared to an antibody consisting of a heavy chain of SEQ ID NO: 9, 29, 49, 69, 89, or 109 and a light chain of SEQ ID NO: 19, 39, 59, 79, 99, or 119.

21. The antibody drug conjugate of any of claims 1-20, wherein said antibody is a human antibody.

22. The antibody drug conjugate of any of claims 1-21, wherein said antibody is a monoclonal antibody.

23. The antibody drug conjugate of any one of claims 1-22, wherein said linker is selected from the group consisting of a cleavable linker, a non-cleavable linker, a hydrophilic linker, a procharged linker and a dicarboxylic acid based linker.

24. The antibody drug conjugate of claim 23, wherein the linker is derived from a cross-linking reagent selected from the group consisting of N-succinimidyl-3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl-4-(2-pyridyldithio)-2-sulfo-butanoate (sulfo-SPDB), N-succinimidyl iodoacetate (SIA), N-succinimidyl(4-iodoacetyl)aminobenzoate (SIAB), maleimide PEG NHS, N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC), N-sulfosuccinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (sulfo-SMCC) or 2,5-dioxopyrrolidin-1-yl 17-(2,5-dioxo-2,5-dihydro-1H-pyrrol-1-yl)-5,8,11,14-tetraoxo-4,7,10,13-tetraazaheptadecan-1-oate (CX1-1).

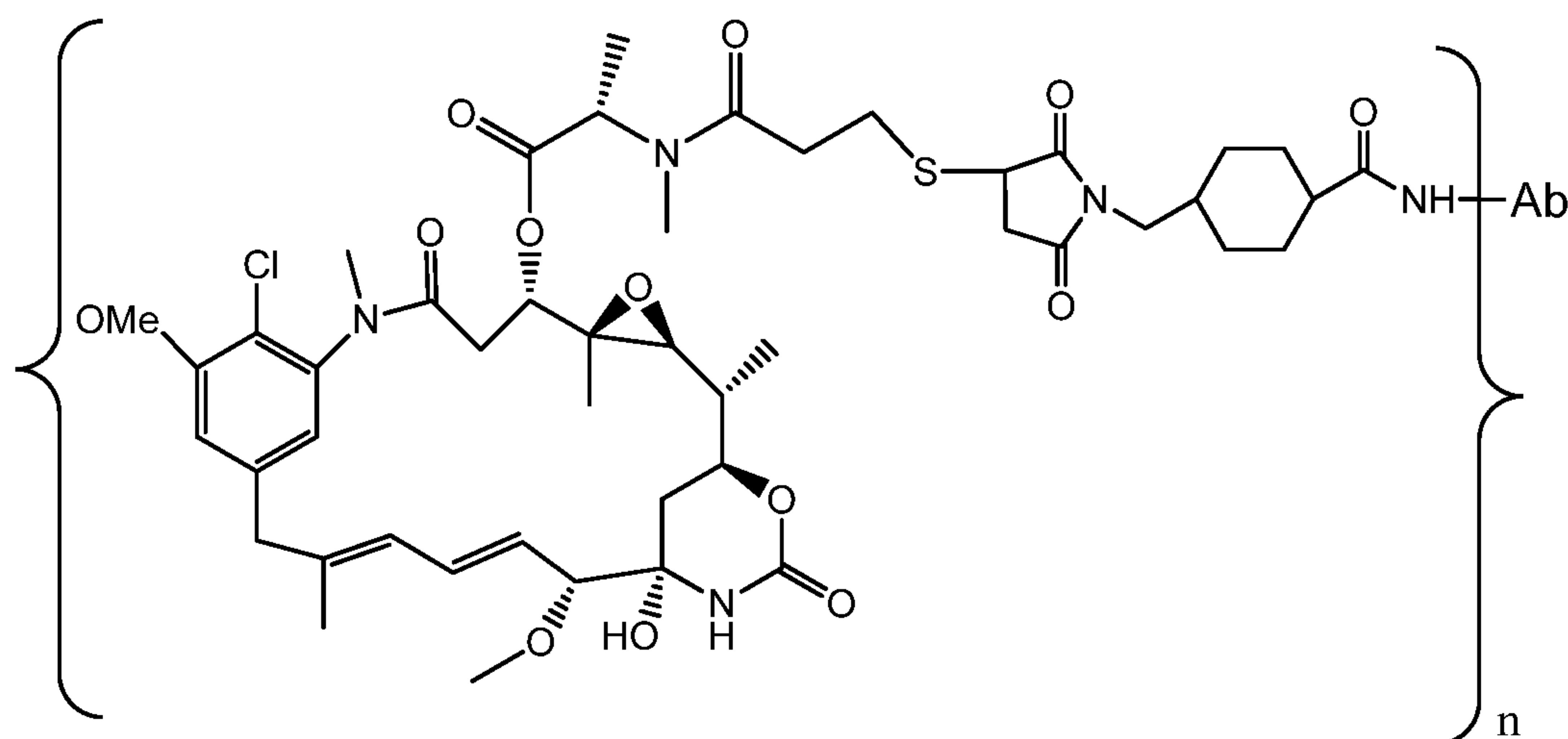
25. The antibody drug conjugate of claim 24, wherein said linker is derived from the cross-linking reagent N-succinimidyl 4-(maleimidomethyl) cyclohexanecarboxylate (SMCC).

26. The antibody drug conjugate of any one of claims 1-25, wherein said drug moiety is selected from a group consisting of a V-ATPase inhibitor, a pro-apoptotic agent, a Bcl2 inhibitor, an MCL1 inhibitor, a HSP90 inhibitor, an IAP inhibitor, an mTor inhibitor, a microtubule stabilizer, a microtubule destabilizer, an auristatin, a dolastatin, a maytansinoid, a MetAP (methionine aminopeptidase), an inhibitor of nuclear export of proteins CRM1, a DPPIV inhibitor, proteasome inhibitors, inhibitors of phosphoryl transfer reactions in mitochondria, a protein synthesis inhibitor, a kinase inhibitor, a CDK2 inhibitor, a CDK9 inhibitor, a kinesin inhibitor, an HDAC inhibitor, a DNA damaging agent, a DNA alkylating agent, a DNA intercalator, a DNA minor groove binder and a DHFR inhibitor.

27. The antibody drug conjugate of claim 26, wherein the cytotoxic agent is a maytansinoid.

28. The antibody drug conjugate of claim 27, wherein the maytansinoid is N(2')- deacetyl-N(2')-(3-mercapto-1-oxopropyl)-maytansine (DM1) or N(2')-deacetyl-N2-(4- mercapto-4-methyl- 1 - oxopentyl)-maytansine (DM4).

29. The antibody drug conjugate of claim 1 having the following formula:



wherein Ab is an antibody or antigen binding fragment thereof comprising a heavy chain CDR1 of SEQ ID NO: 1, 21, 41, 61, 81, or 101, a heavy chain CDR2 of SEQ ID NO: 2, 22, 42, 62, 82, or 102, a heavy chain CDR3 of SEQ ID NO: 3, 23, 43, 63, 83, or 103 and a light chain CDR1 of SEQ ID NO: 11, 31, 51, 71, 91, or 111 a light chain CDR2 of SEQ ID NO: 12, 32, 52, 72, 92, or 112 a light chain CDR3 of SEQ ID NO: 13, 33, 53, 73, 93, or 113 wherein the CDR is defined in accordance with the Kabat definition; and n is 1 to 10.

30. A pharmaceutical composition comprising the antibody drug conjugate of any one of claims 1-29 and a pharmaceutically acceptable carrier.
31. The pharmaceutical composition of claim 30 wherein said composition is prepared as a lyophilisate.
32. The pharmaceutical composition of claim 31, wherein said lyophilisate comprises said antibody drug conjugate, sodium succinate, and polysorbate 20.
33. A method of treating an FGFR2 positive and/or FGFR4 positive cancer in a patient in need thereof, comprising administering to said patient the antibody drug conjugate of any of claims 1-30, or the pharmaceutical composition of any of claims 30-32.
34. The method of claim 29, wherein the cancer has an increase in FGFR2 gene copy number or the PAX3-FOXO translocation.
35. The method of claim 33, wherein said cancer is selected from the group consisting of gastric cancer, breast cancer, rhabdomyosarcoma, liver cancer, adrenal cancer, lung cancer, esophageal cancer, colon cancer and endometrial cancer.
36. The method of claim 33 or 34 further comprising administering to said patient a tyrosine kinase inhibitor, an IAP inhibitor, a Bcl2 inhibitor, an MCL1 inhibitor, or another FGFR2 inhibitor.
37. The method of claim 36, wherein said another FGFR2 inhibitor is 3-(2,6-Dichloro-3,5-dimethoxy-phenyl)-1-{6-[4-(4-ethyl-piperazin-1-yl)-phenylamino]-pyrimidin-4-yl}-1-methyl-urea or a pharmaceutically acceptable salt thereof.
38. The method of claim 33 or 35, wherein the cancer is resistant to a tyrosine kinase inhibitor.
39. The method of claim 38, wherein the tyrosine kinase inhibitor is an EGFR, Her2, Her3, IGFR or Met inhibitor.
40. A method of treating cancer resistant to Her2 inhibitors in a patient, comprising administering to said patient the antibody drug conjugate of any of claims 1-29, or the pharmaceutical composition of any of claims 30-32.
41. The antibody drug conjugate of any of claims 1-29 for use as a medicament.

42. The antibody drug conjugate of any of claims 1-29, or pharmaceutical composition of any of claims 31-33, for use in the treatment of an FGFR2 positive or FGFR4 positive cancer.

43. The antibody drug conjugate of any of claims 1-29, or pharmaceutical composition of any of claims 31-33, for use in the treatment of cancer resistant to tyrosine kinase inhibitors.

44. The antibody drug conjugate of any of claims 1-29, or pharmaceutical composition of any of claims 30-32, for use in the treatment of cancer resistant to EGFR, Her2, Her3, IGFR or Met inhibitors.

45. An antibody or antigen binding fragment thereof that specifically binds to FGFR2 and FGFR4.

46. The antibody or antigen binding fragment of claim 45, wherein said antibody or antigen binding fragment specifically binds to an epitope on human FGFR2 comprising amino acid residues 176 (Lys) and 210 (Arg) of SEQ ID NO:137.

47. The antibody or antigen binding fragment of claim 46, wherein said antibody or antigen binding fragment specifically binds amino acid residues 173 (Asn), 174 (Thr), 175 (Val), 176 (Lys), 178 (Arg), 208 (Lys), 209 (Val), 210 (Arg), 212 (Gln), 213 (His), 217 (Ile), and 219 (Glu) of SEQ ID NO:137.

48. The antibody or antigen binding fragment of claim 47, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR2 comprising SEQ ID NO:136 or SEQ ID NO:141.

49. The antibody or antigen binding fragment of claim 46-48, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR4 comprising amino acid residues 169 (Lys) and 203 (Arg) of SEQ ID NO: 142.

50. The antibody or antigen binding fragment of claim 45, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR2 comprising amino acid residues 176 (Lys) and 210 (Arg) of SEQ ID NO:137 and an epitope of human FGFR4 comprising amino acid residues 169 (Lys) and 203 (Arg) of SEQ ID NO: 142.

51. The antibody or antigen binding fragment any of claims 45-50, wherein said antibody or antigen binding fragment specifically binds amino acid residues 150 (Thr), 151 (His), 154 (Arg), 157 (Lys), 160 (His), 166 (Asn), 167 (Thr), 168 (Val), 169 (Lys), 171 (Arg), 173 (Pro), 174 (Ala),

201 (Arg), 202 (Leu), 203 (Arg), 204 (His), 205 (Gln), 206 (His), 207 (Trp), 210 (Val) and 212 (Glu) of SEQ ID NO:142.

52. The antibody or antigen binding fragment of any of claims 45-51, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR4 comprising SEQ ID NO: 132 or 133.

53. The antibody or antigen binding fragment any of claims 45-51, wherein said antibody or antigen binding fragment specifically binds to an epitope of human FGFR4 consisting of SEQ ID NO: 132 or 133.

54. The antibody or antigen binding fragment of any of claims 45-53 comprising a heavy chain variable region that comprises: (a) a VH CDR1 of SEQ ID NO: 1, 21, 41, 61, 81, or 101, (b) a VH CDR2 of SEQ ID NO: 2, 22, 42, 62, 82 or 102 and (c) a VH CDR3 of SEQ ID NO: 3, 23, 43, 63, 83, or 103 wherein the CDR is defined in accordance with the Kabat definition.

55. The antibody or antigen binding fragment of claim 54 further comprising a light chain variable region that comprises: (a) a VL CDR1 of SEQ ID NO: 11, 31, 51, 71, 91, or 111, (b) a VL CDR2 of SEQ ID NO: 12, 32, 52, 72, 92, or 112 and a VL CDR3 of SEQ ID NO: 13, 33, 53, 73, 93, or 113 wherein the CDR is defined in accordance with the Kabat definition.

56. The antibody or antigen binding fragment of claim 55 comprising a VH region of SEQ ID NO: 7, 27, 47, 67, 87, or 107 and a VL region of SEQ ID NO: 17, 37, 57, 77, 97, or 117.

57. The antibody of claim 56 consisting of a heavy chain of SEQ ID NO: 9, 29, 49, 69, 89, or 109 and a light chain of SEQ ID NO: 19, 39, 59, 79, 99, or 119.

58. The antibody or antigen binding fragment of claim 45, wherein said antibody or antigen binding fragment cross competes with the antibody or antigen binding fragment of claim 57 for binding to human FGFR2 and FGFR4.

59. The antibody or antigen binding fragment of claim 45, wherein said antibody or antigen binding fragment has enhanced ADCC activity as compared to the antibody of claim 59.

60. The antibody or antigen binding fragment of any of claims 45 -59, wherein said antibody is a human antibody.

61. The antibody or antigen binding fragment of any of claims 45-60, wherein said antibody is a monoclonal antibody.
62. the antibody or antigen binding fragment of any of claims 45-61, wherein said antibody or antigen binding fragment is a single chain antibody (scFv).
63. A nucleic acid that encodes the antibody or antigen binding fragment of any one of claims any of the preceeding claims.
64. A vector comprising the nucleic acid of claim 63.
65. A host cell comprising the vector according to claim 64.
66. A process for producing an antibody or antigen binding fragment comprising cultivating the host cell of claim 65 and recovering the antibody from the culture.
67. A process for producing an anti-FGFR2 and FGFR4 antibody drug conjugate comprising:
(a) chemically linking SMCC to a drug moiety DM-1;
(b) conjugating said linker-drug to the antibody recovered from the cell culture of claim 66; and
(c) purifying the antibody drug conjugate.
68. The antibody drug conjugate made according to claim 67 having an average DAR, measured with a UV spectrophotometer, about 3.5.
69. A diagnostic reagent comprising the antibody or antigen binding fragment thereof of any one of claims 45-62, or the antibody drug conjugate of any one of claims 1-29 which is labeled.
70. The diagnostic reagent of claim 69, wherein the label is selected from the group consisting of a radiolabel, a fluorophore, a chromophore, an imaging agent, and a metal ion.

FIG. 1

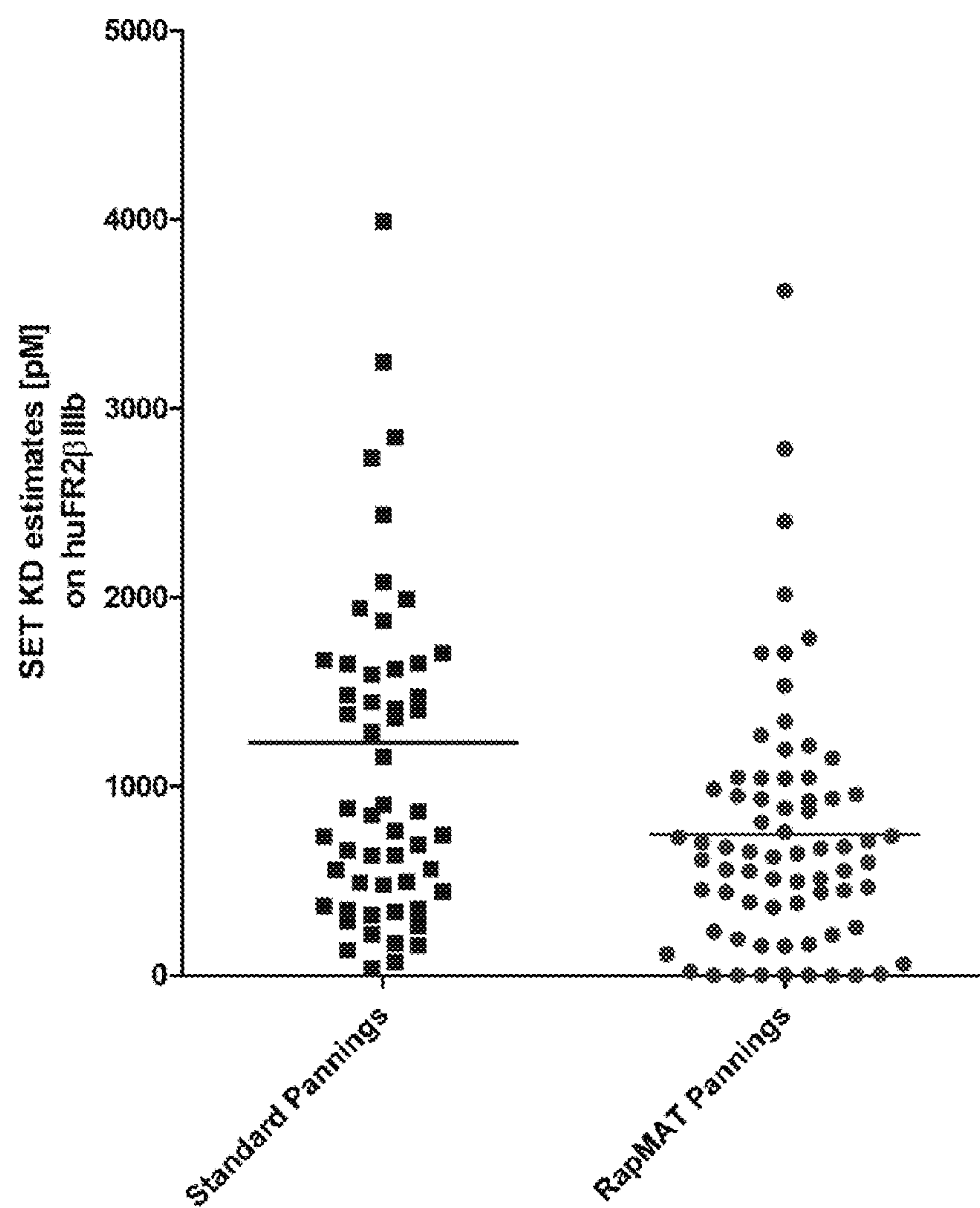
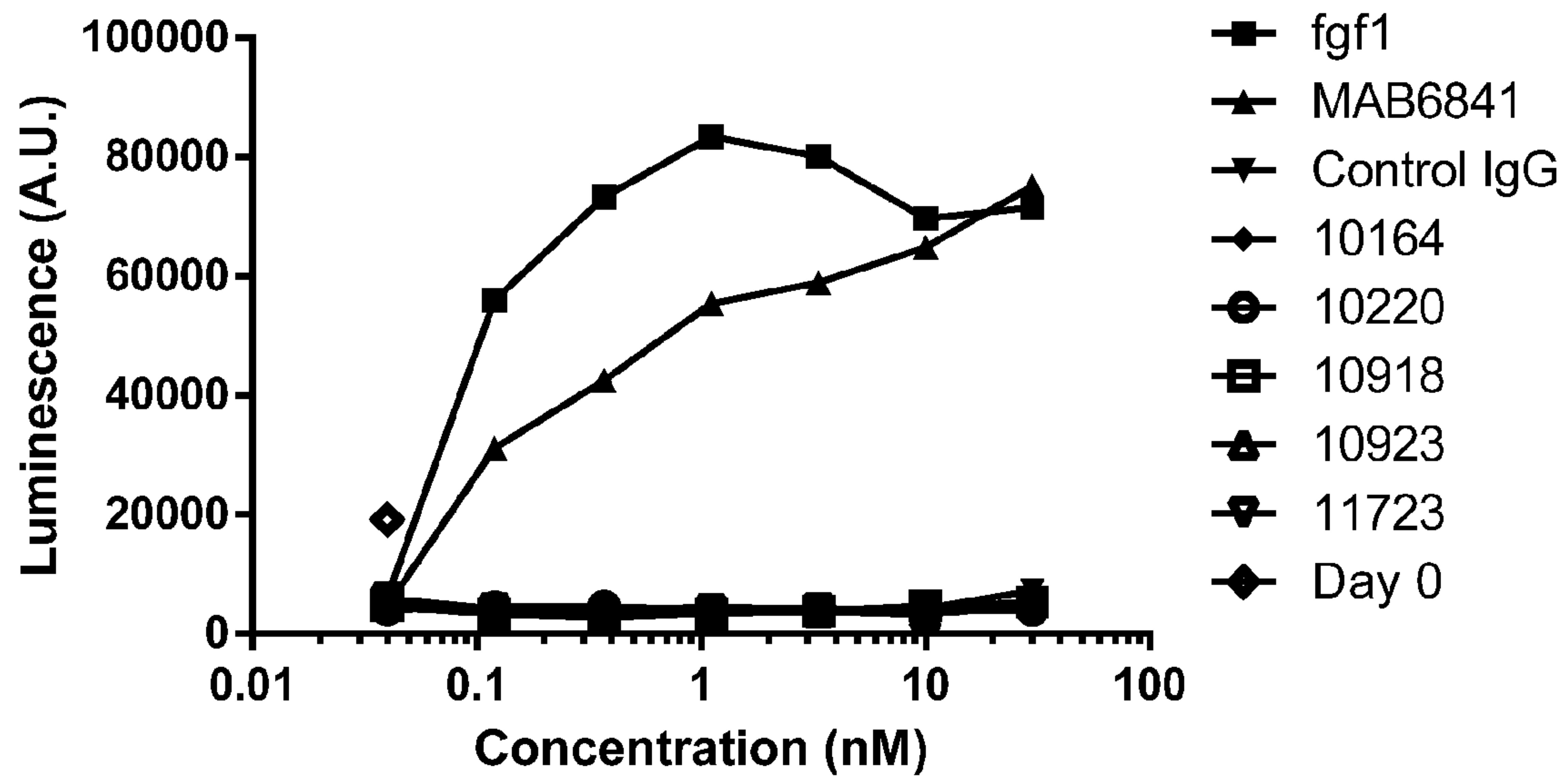


FIG. 2

(A)



(B)

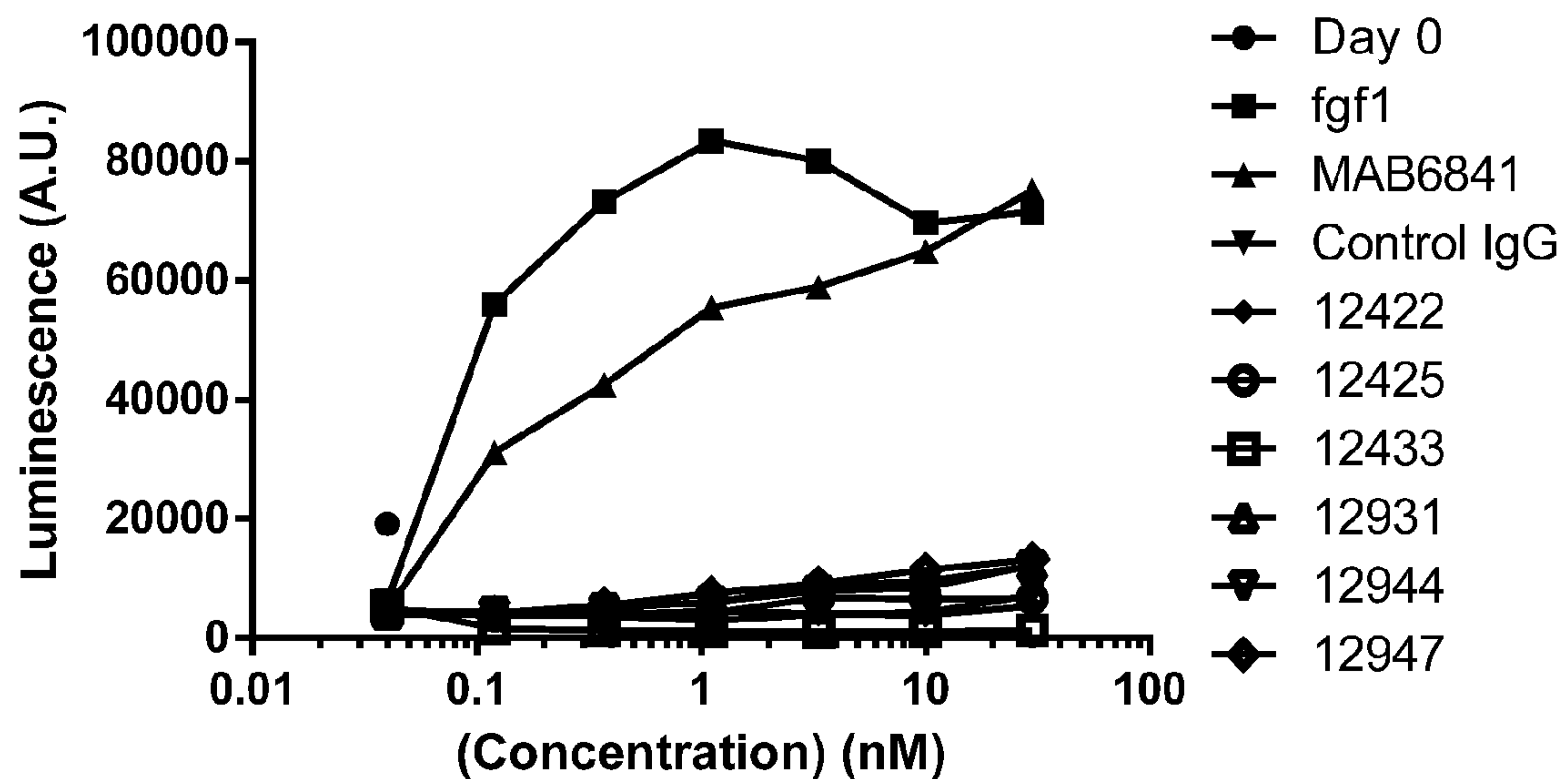
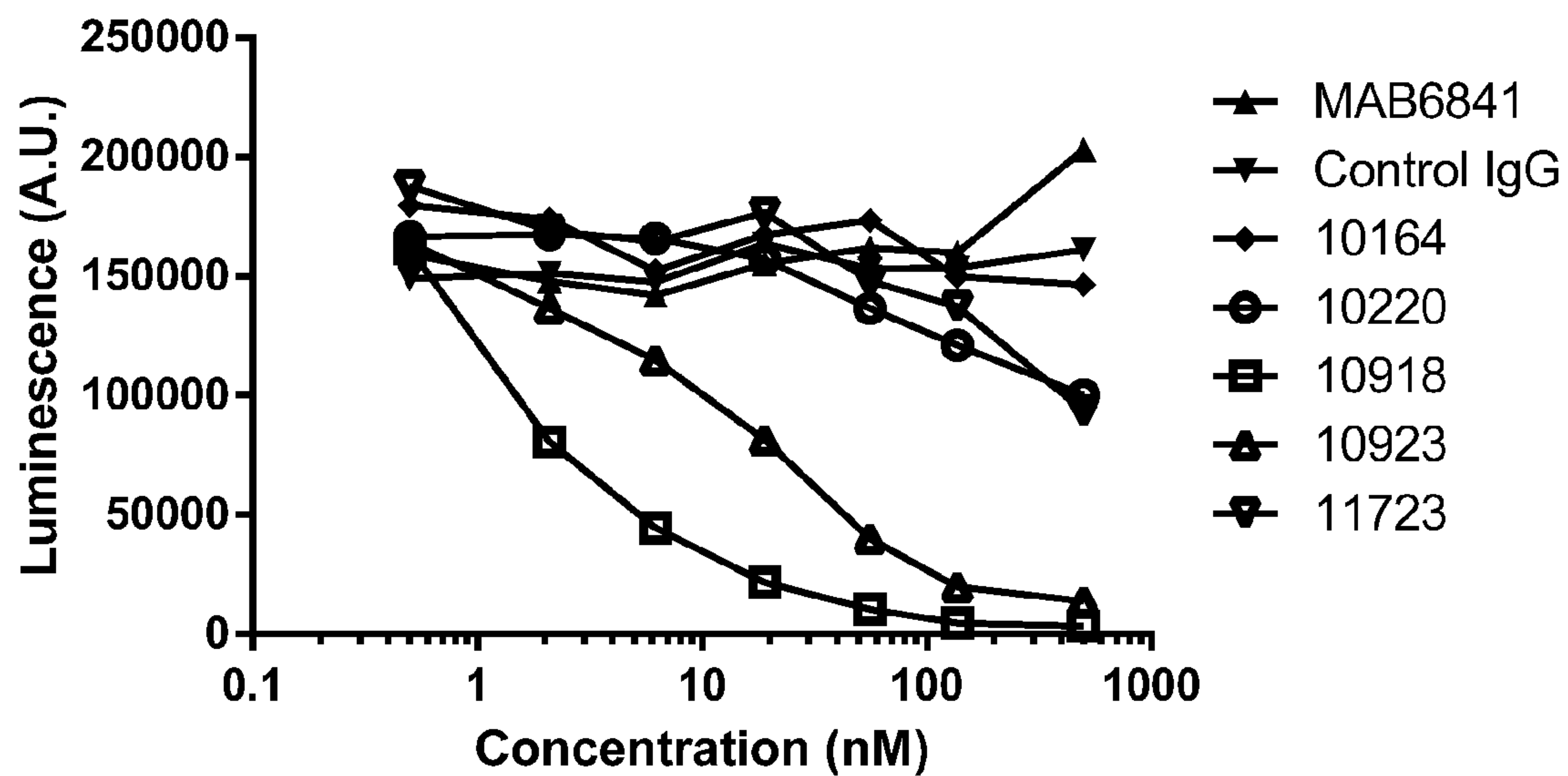


FIG. 3

(A)



(B)

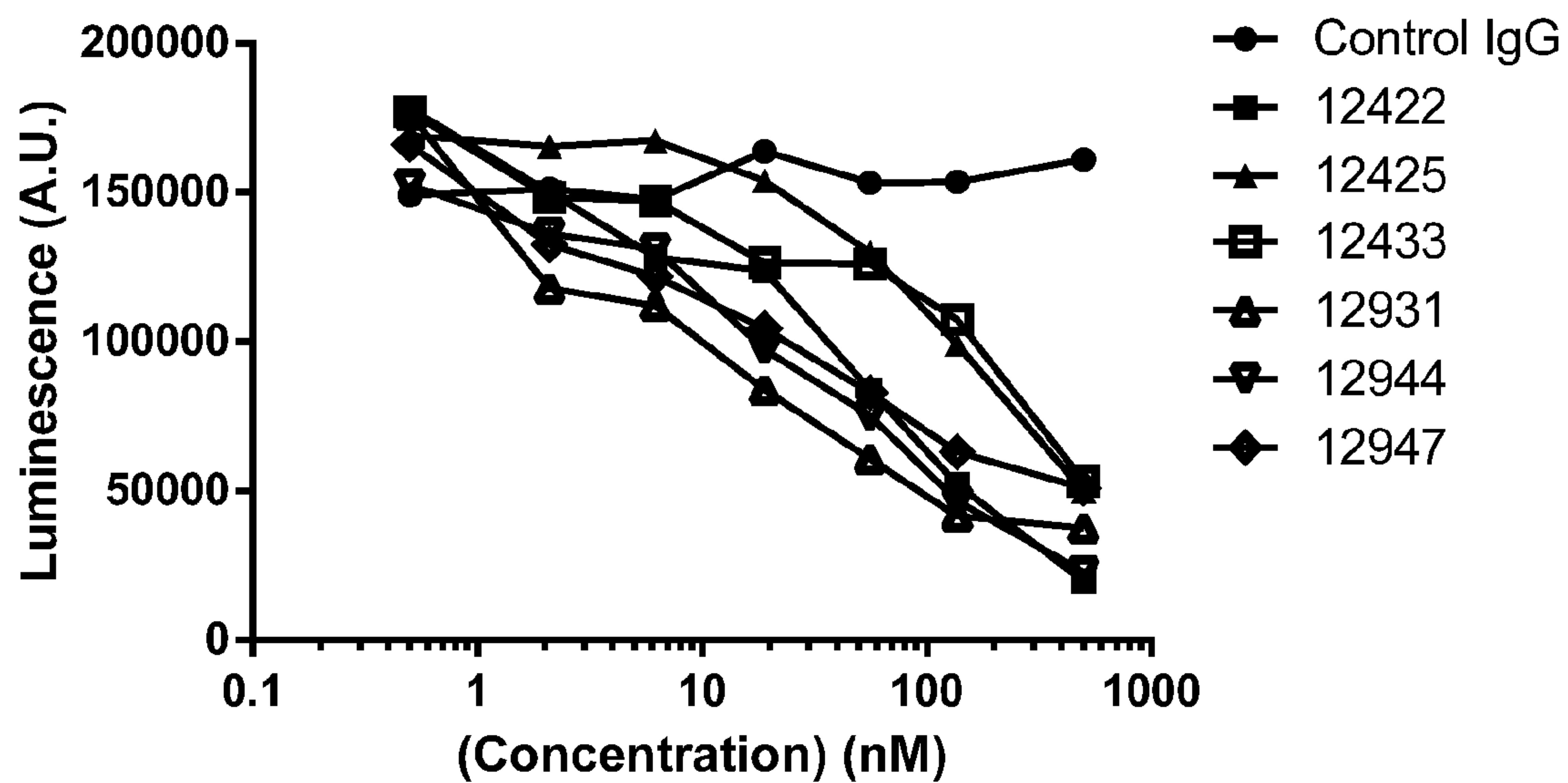


FIG. 4

(A)

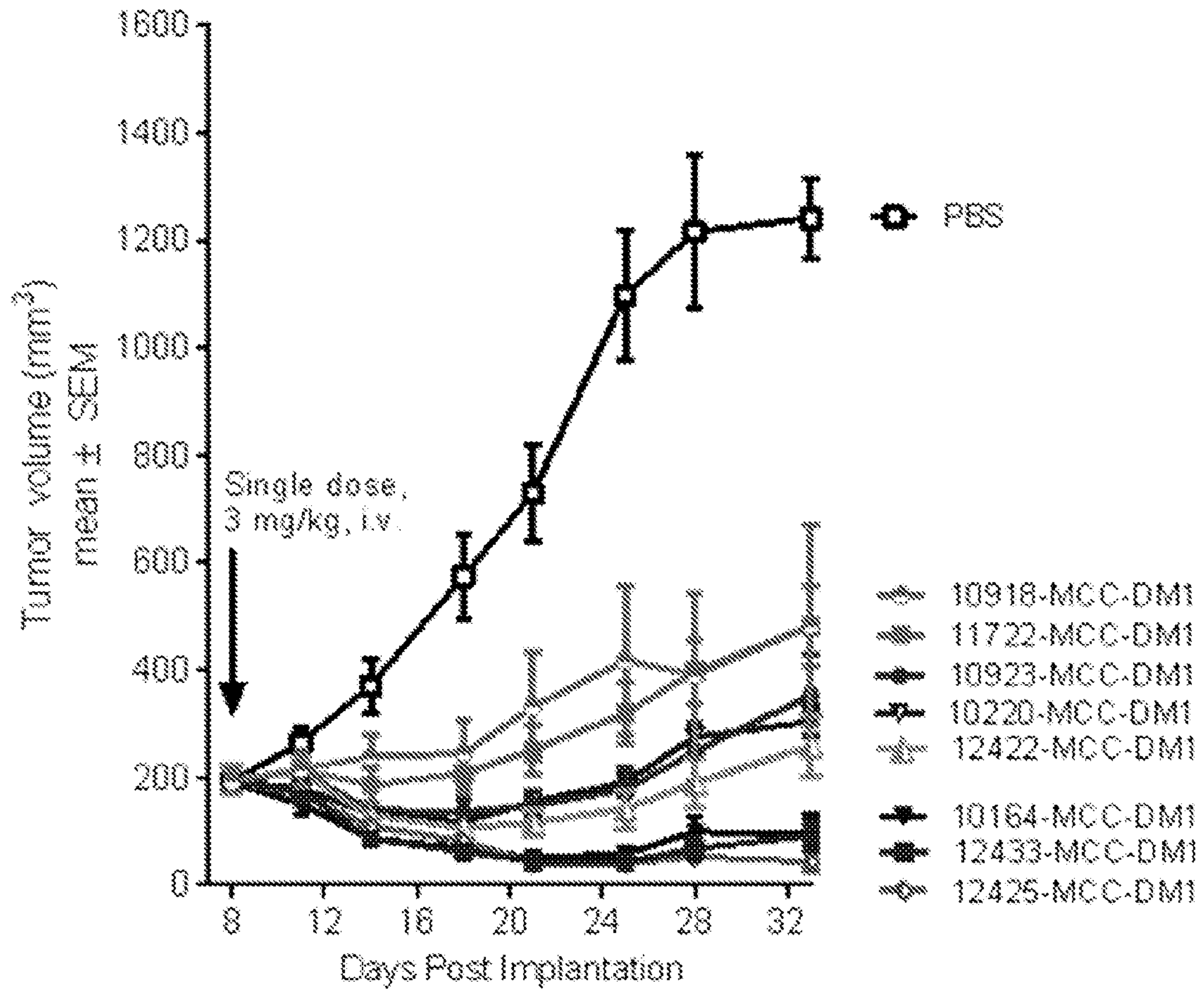


FIG. 4

(B)

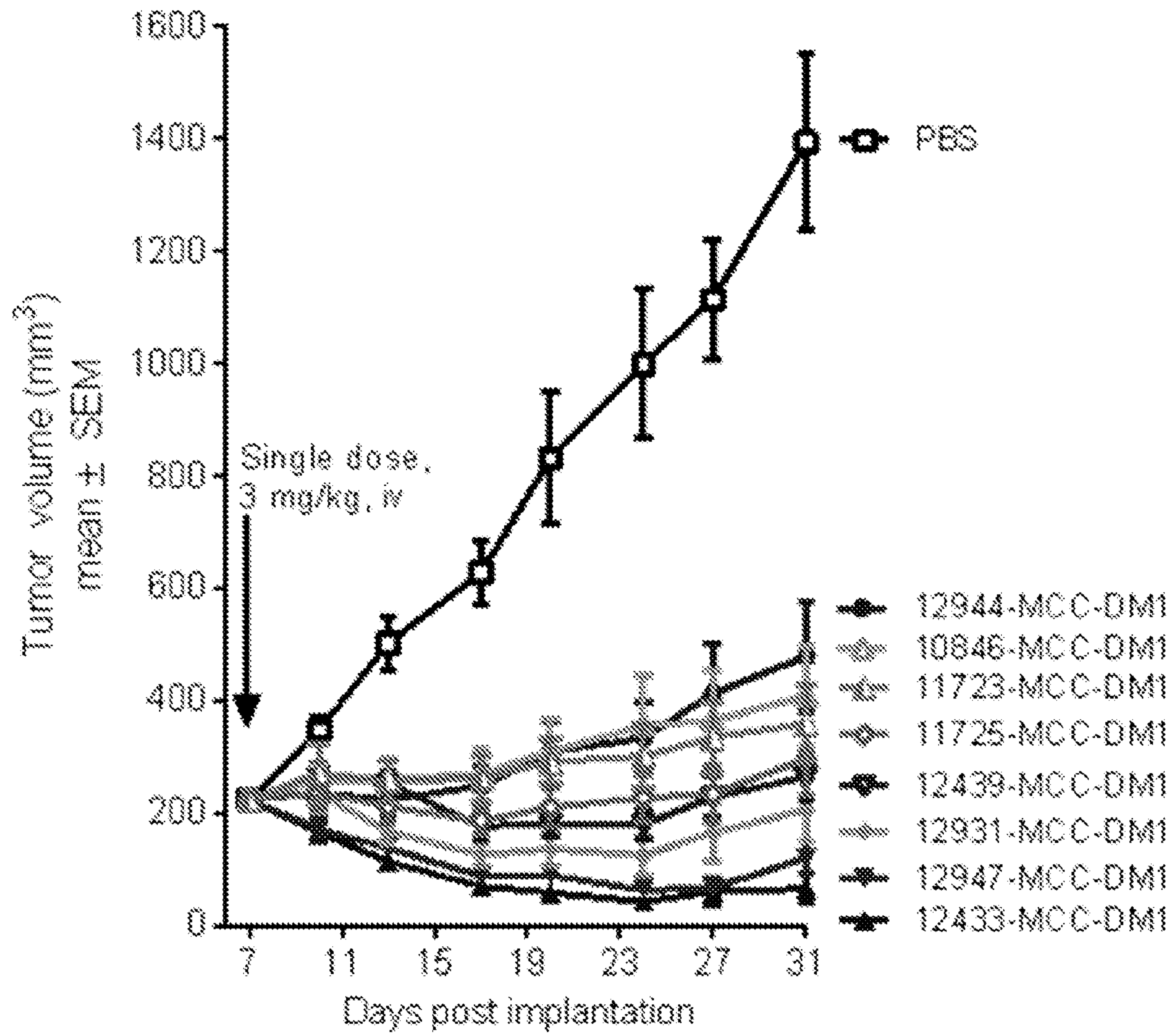
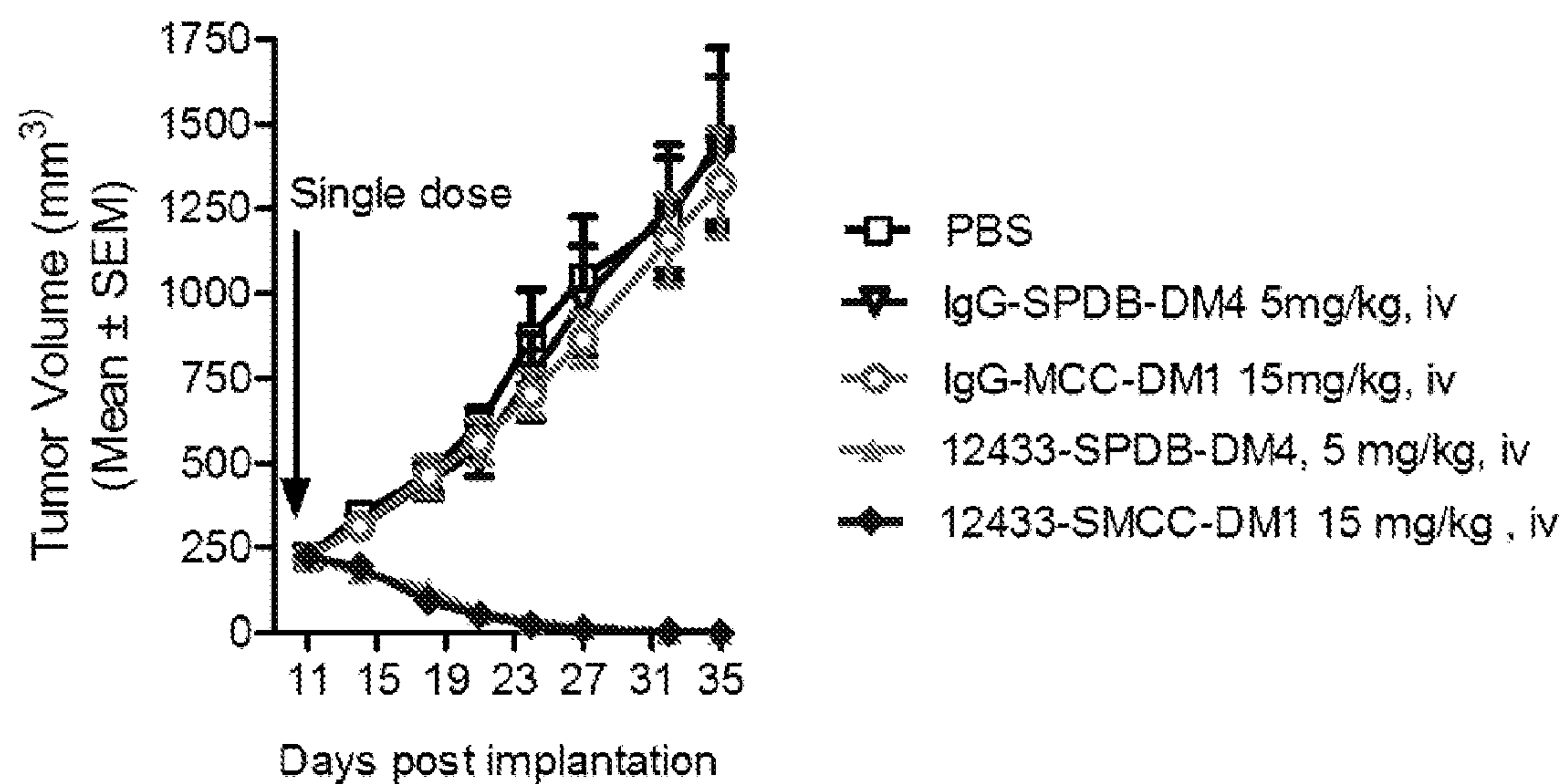
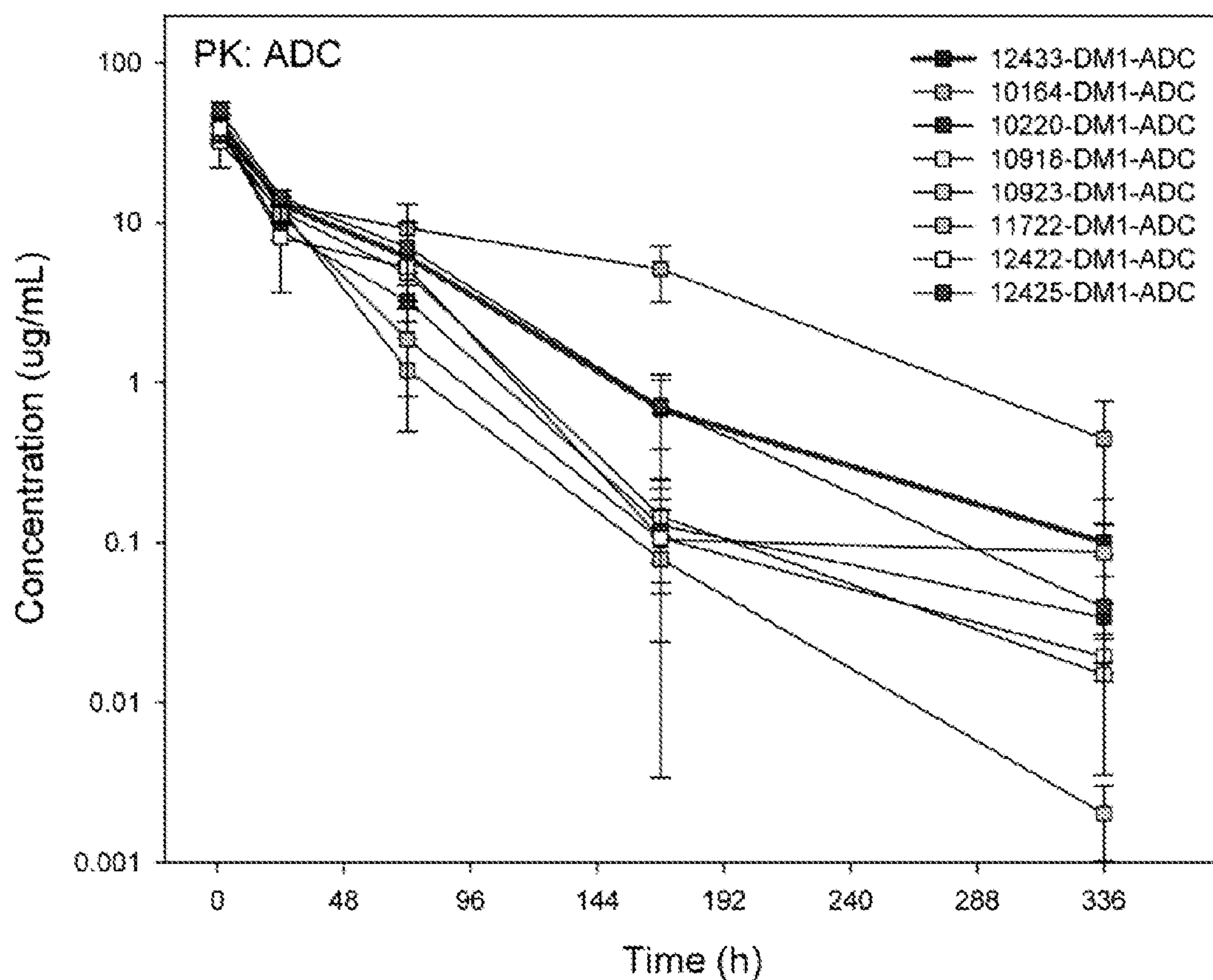


FIG. 4

(C)



(D)



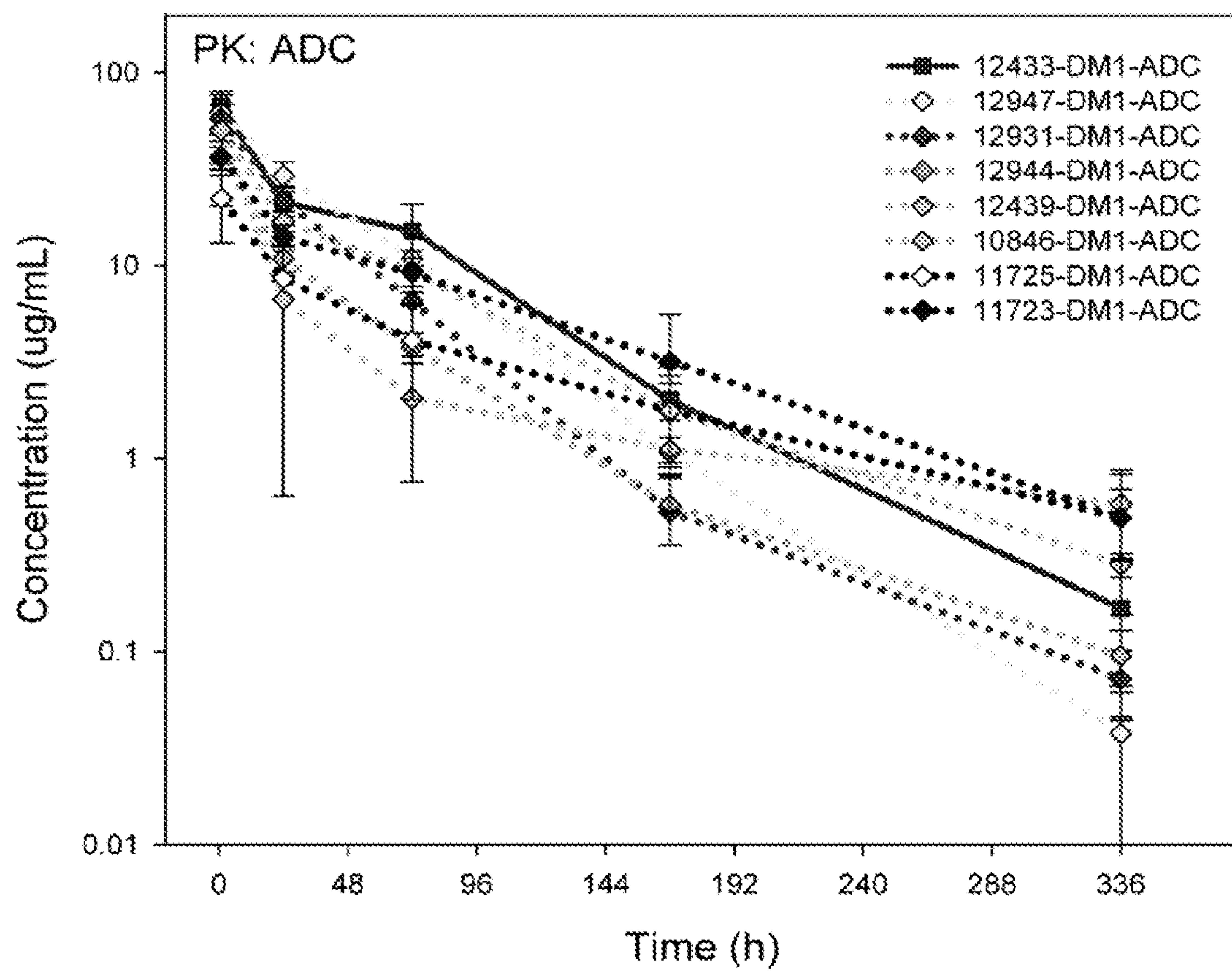


FIG. 4

(F)

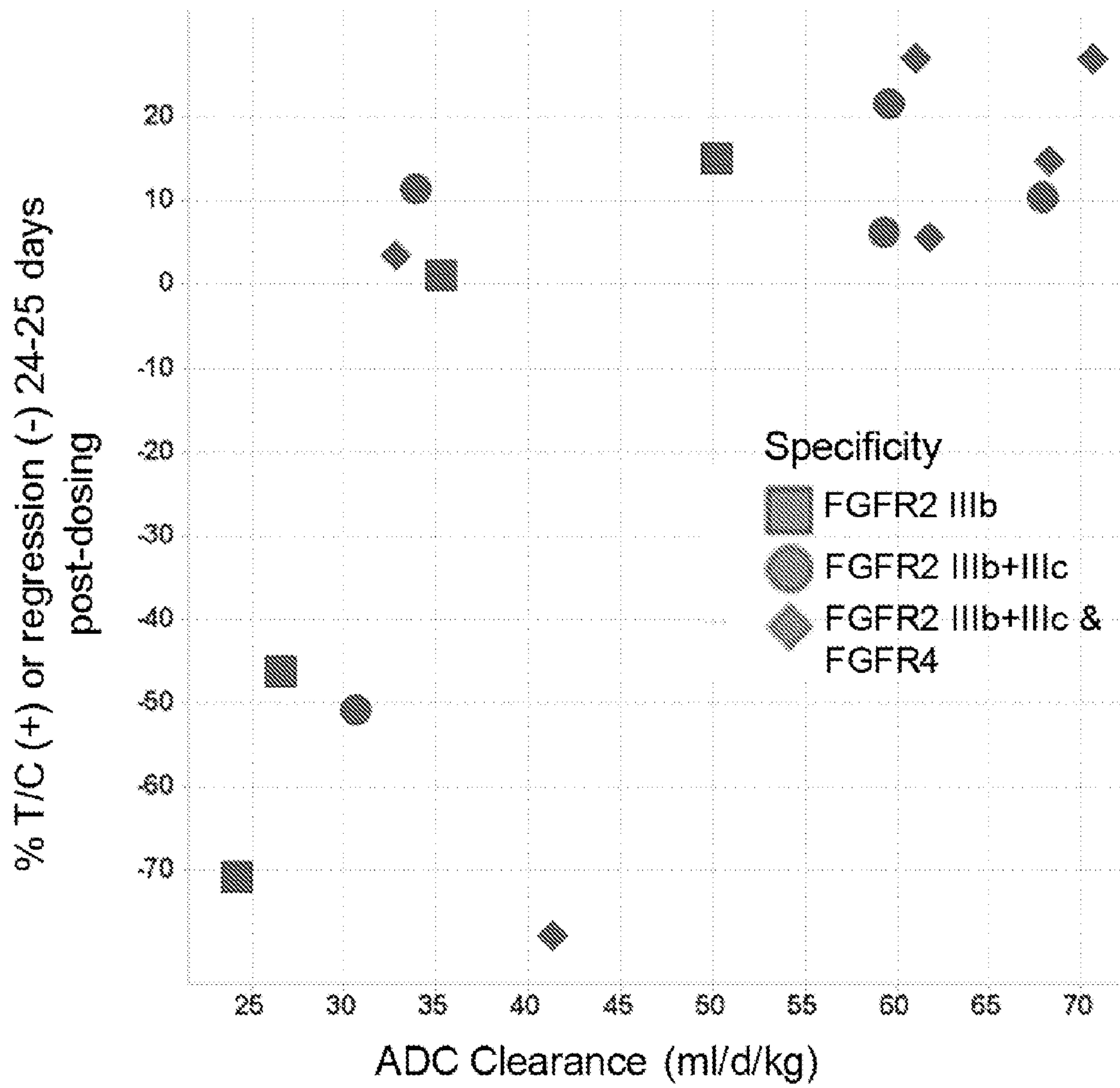
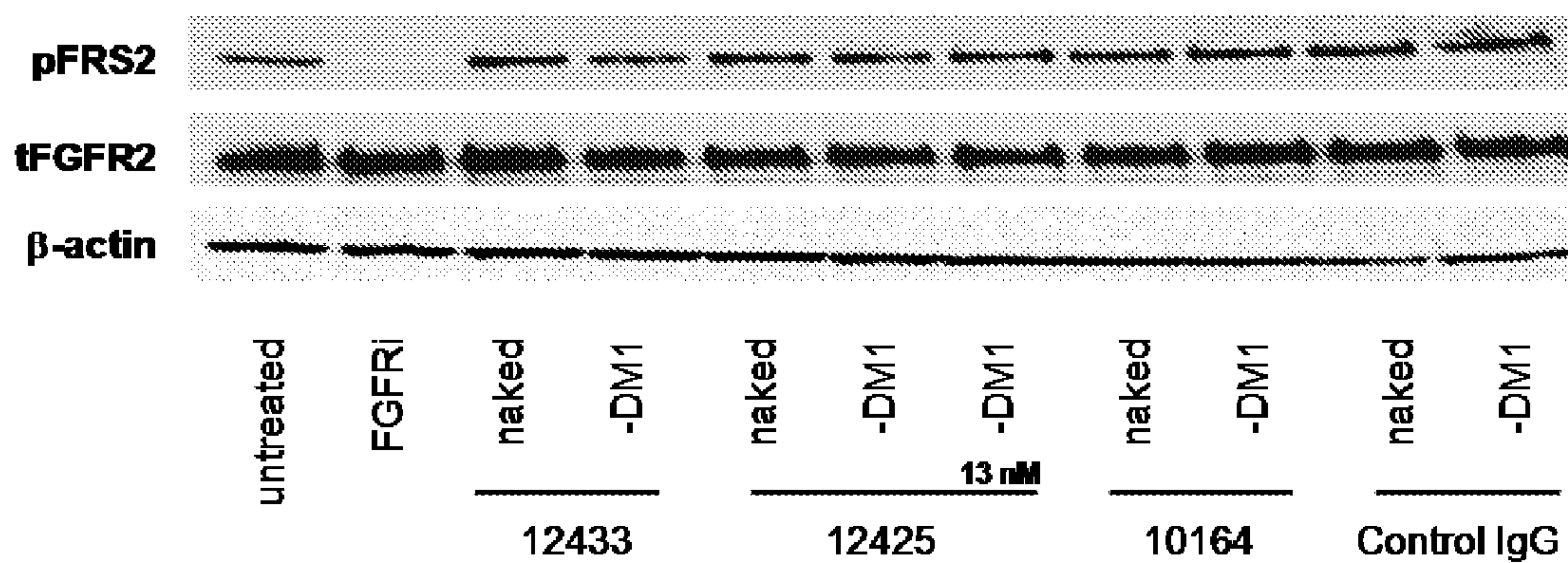
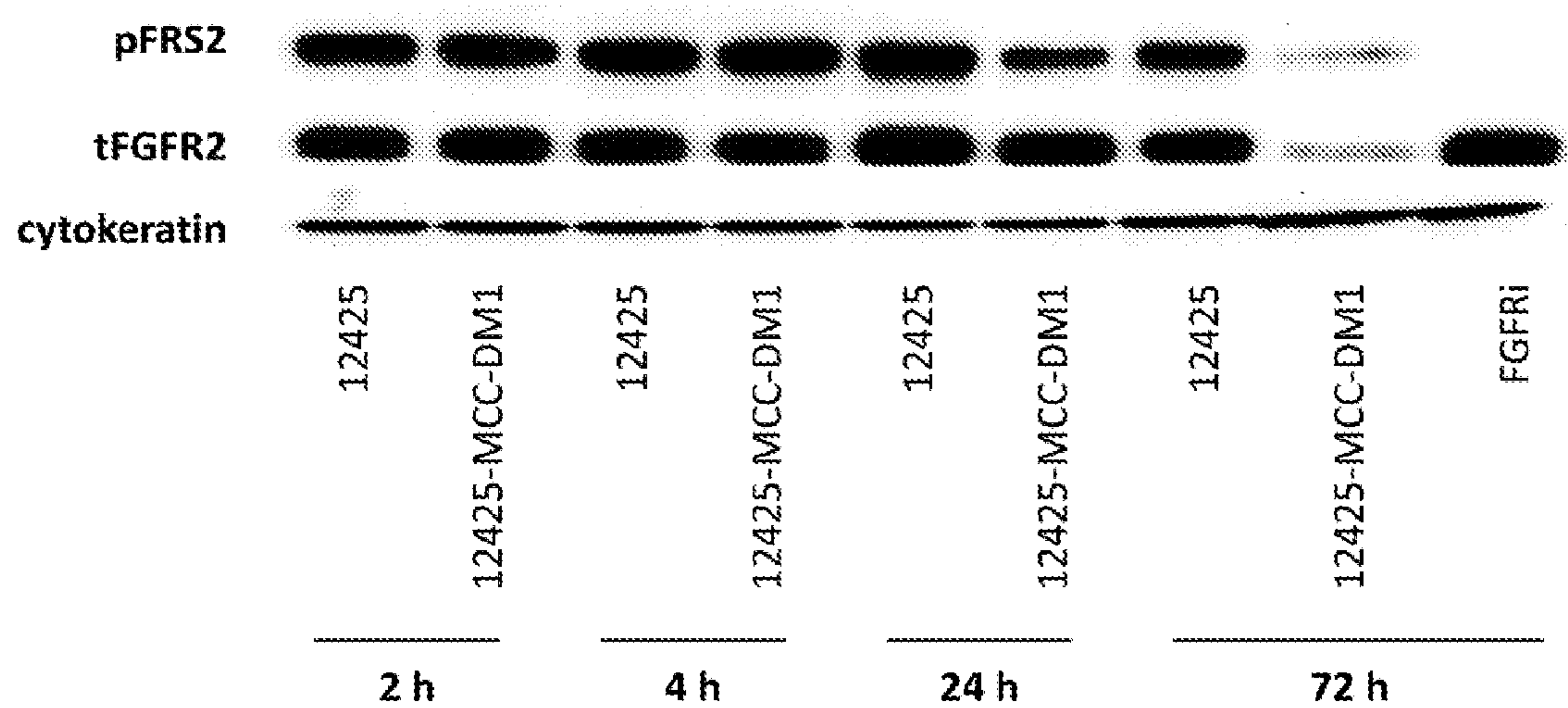


FIG. 5

(A)



(B)



(C)

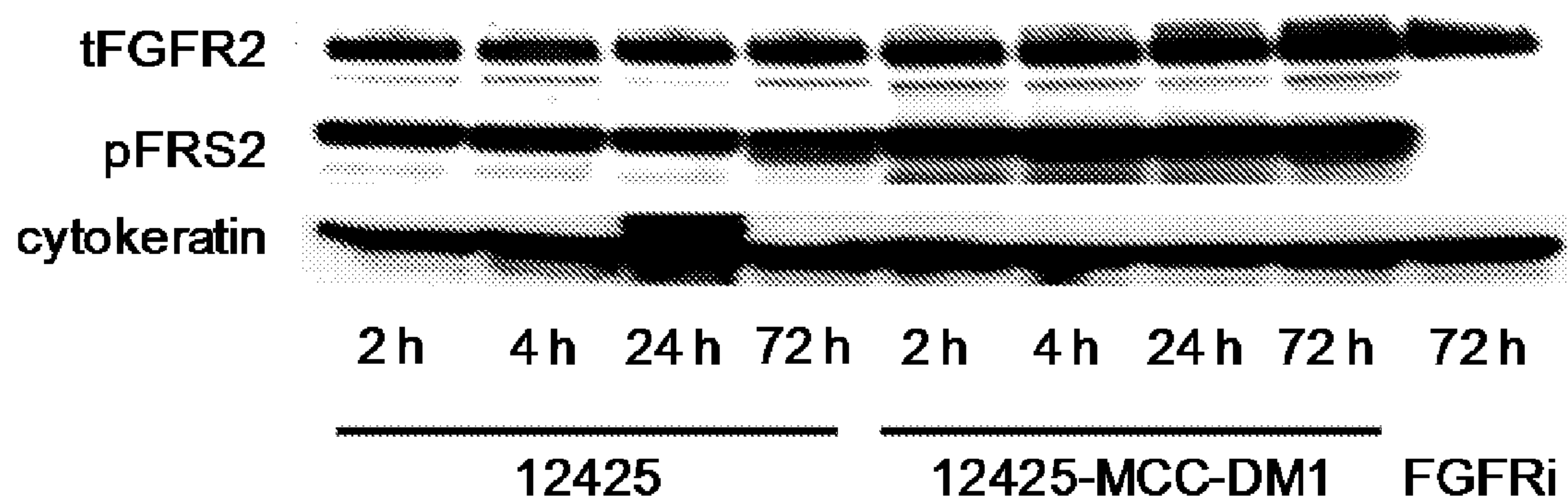
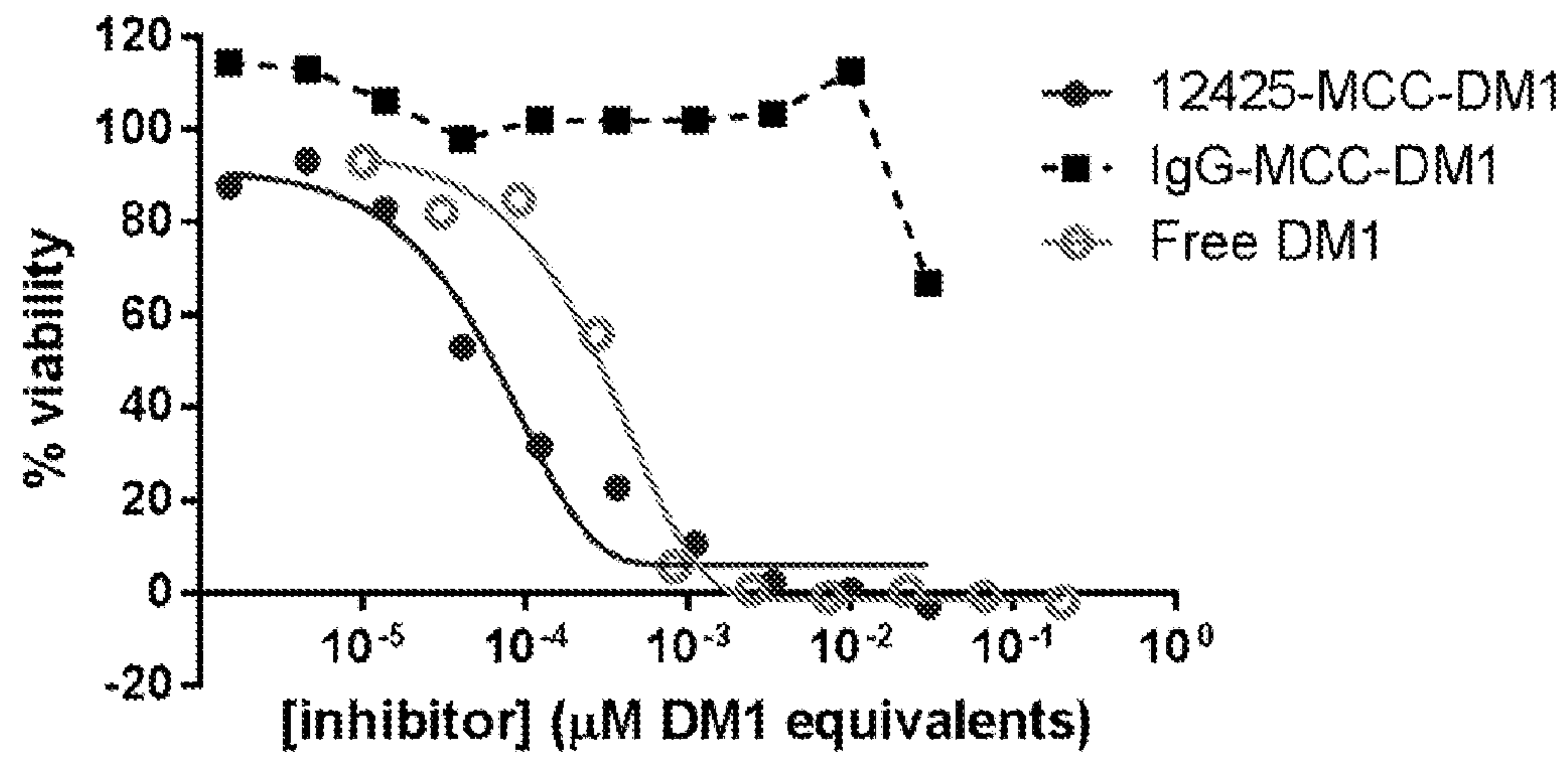


FIG. 6

(A)



(B)

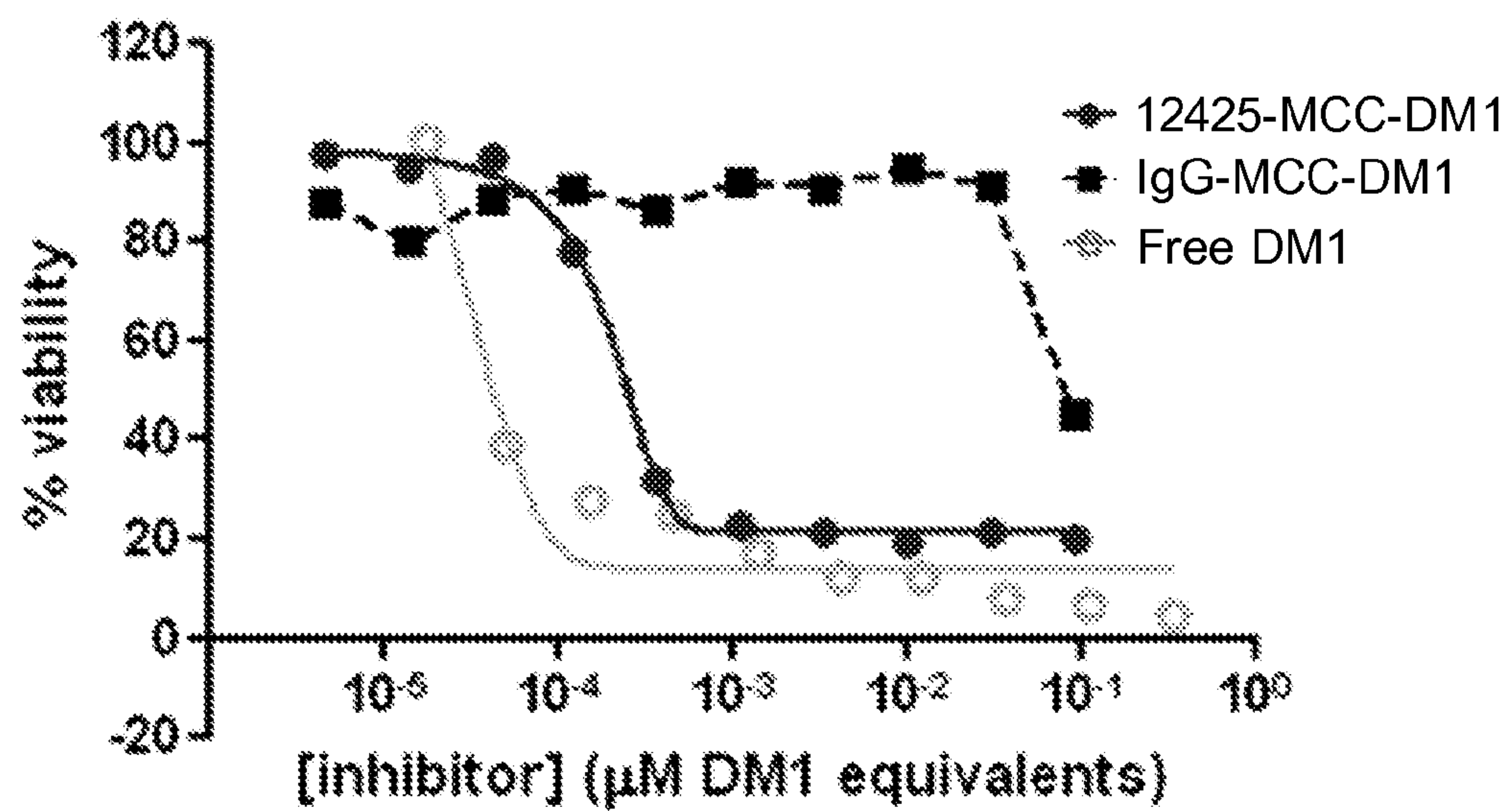
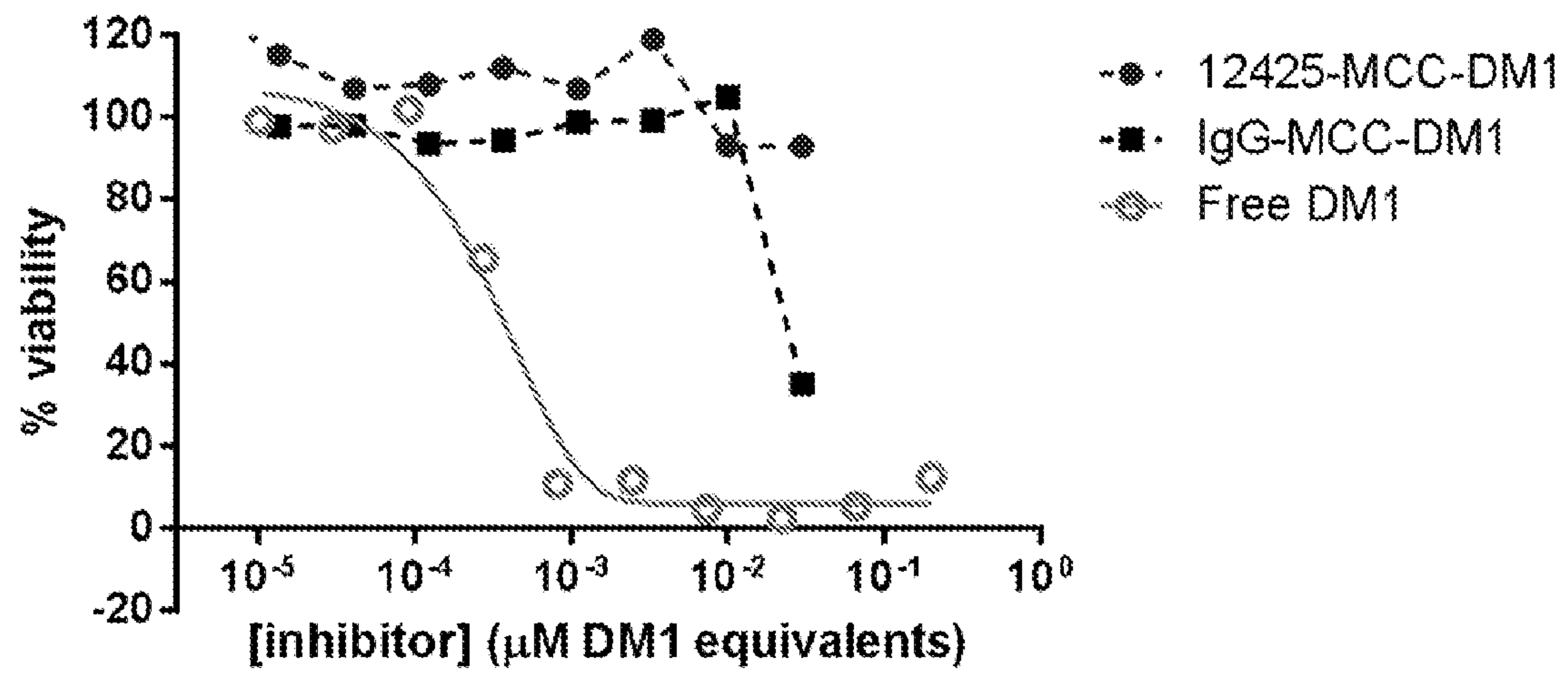
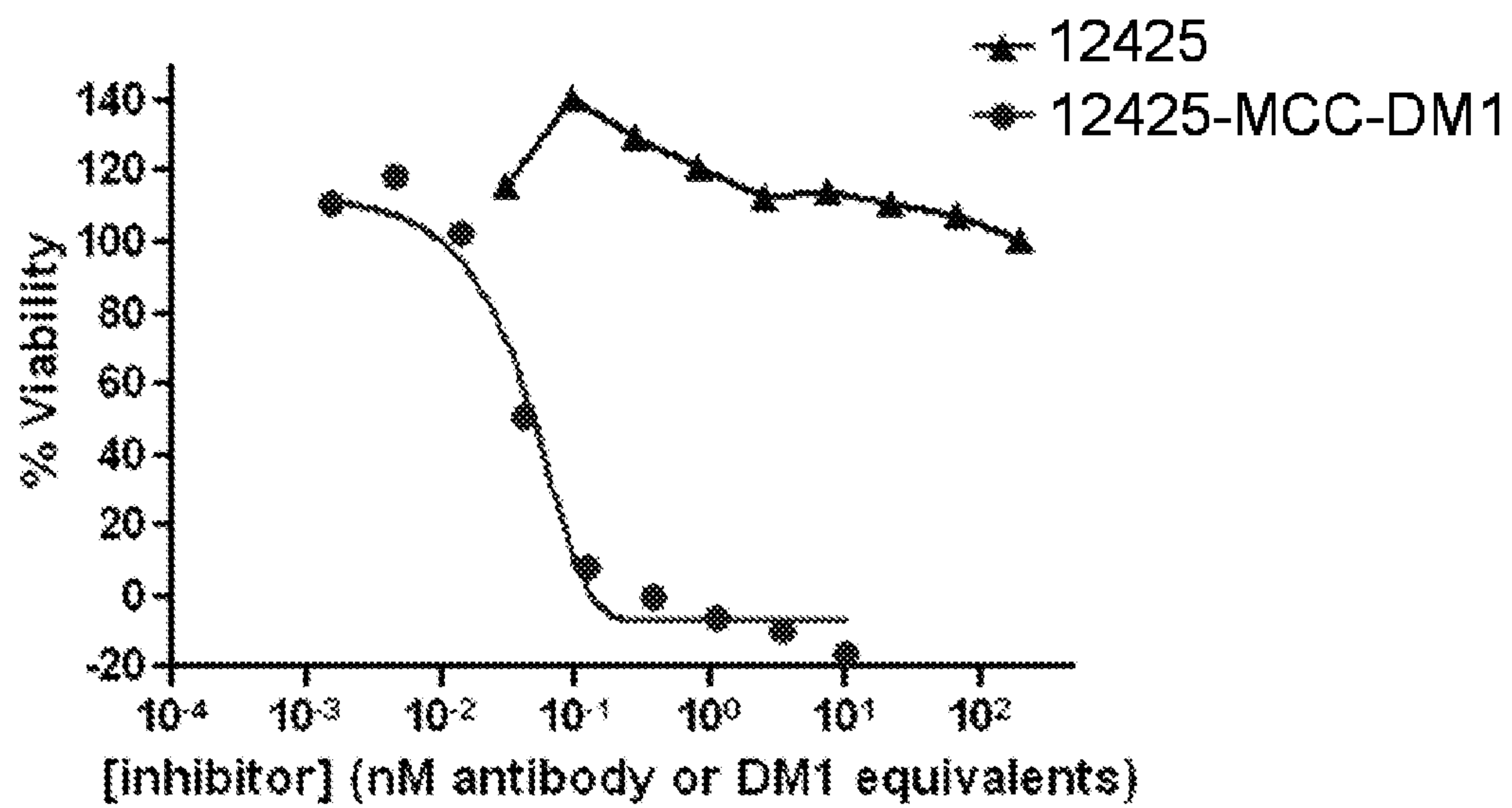


FIG. 6

(C)



(D)



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FIG. 7

(A)

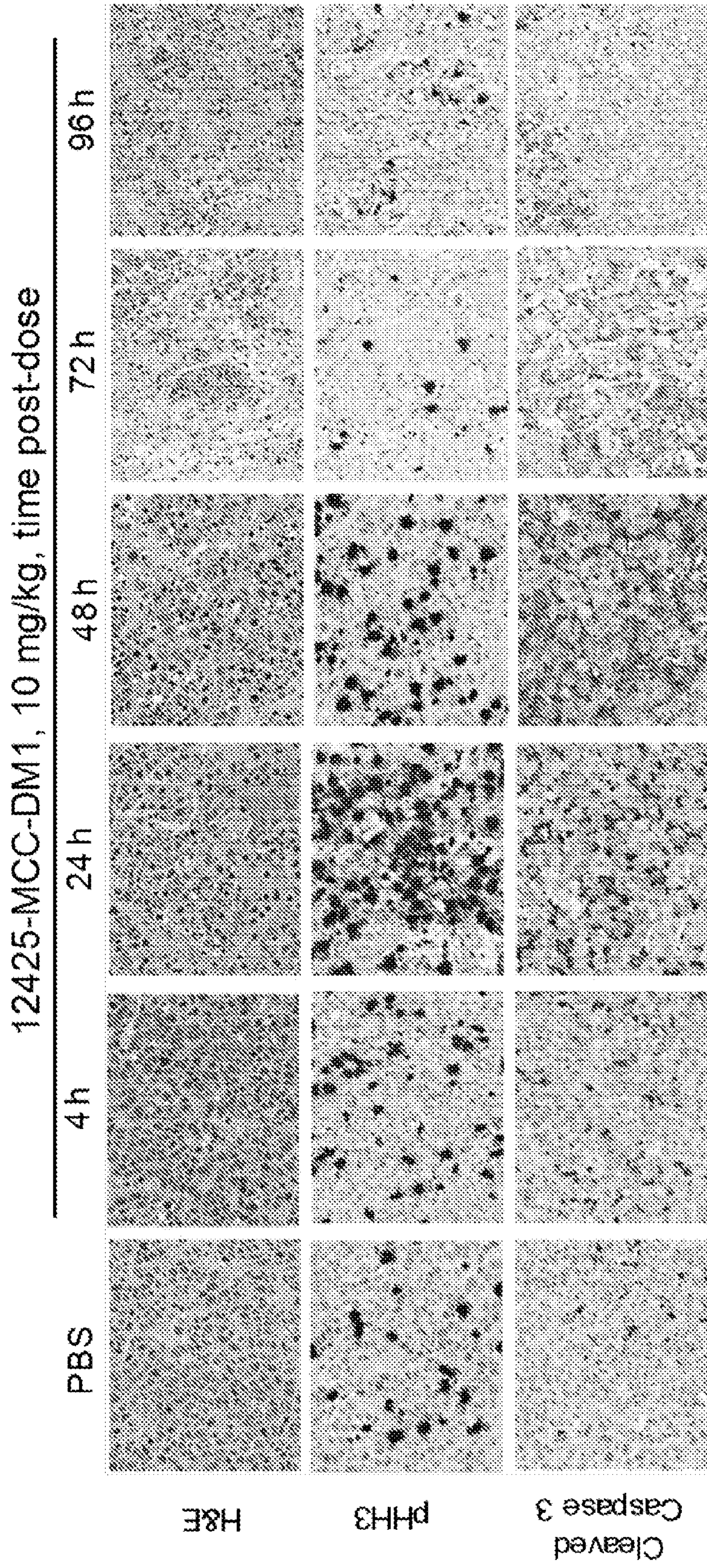


FIG. 7

(B)

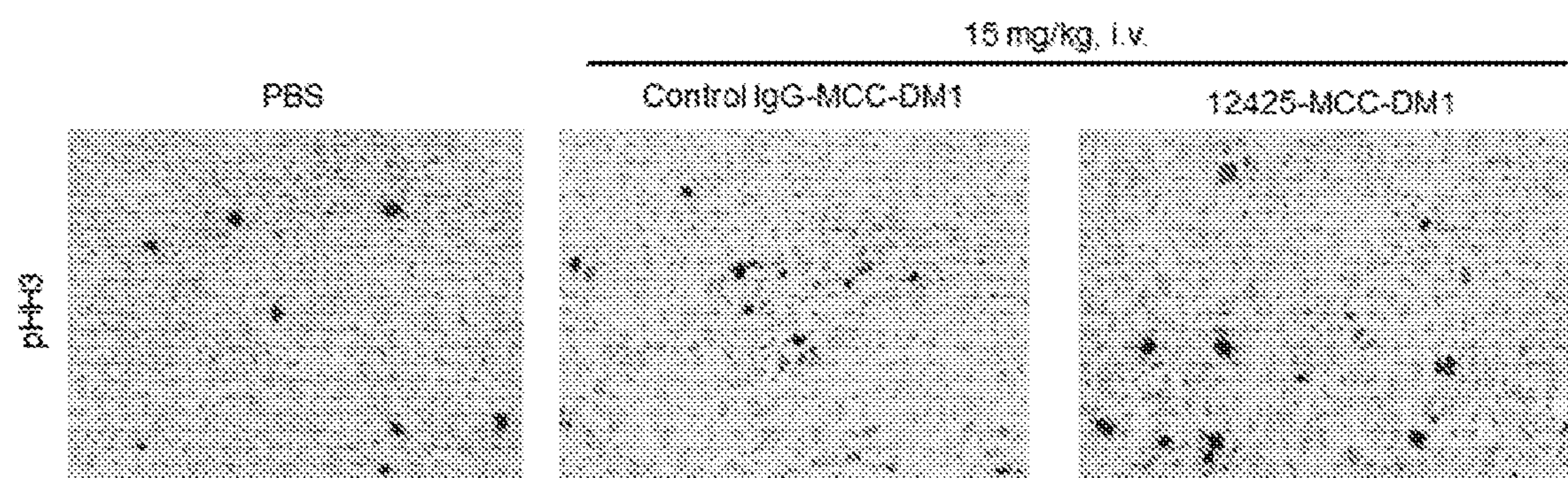
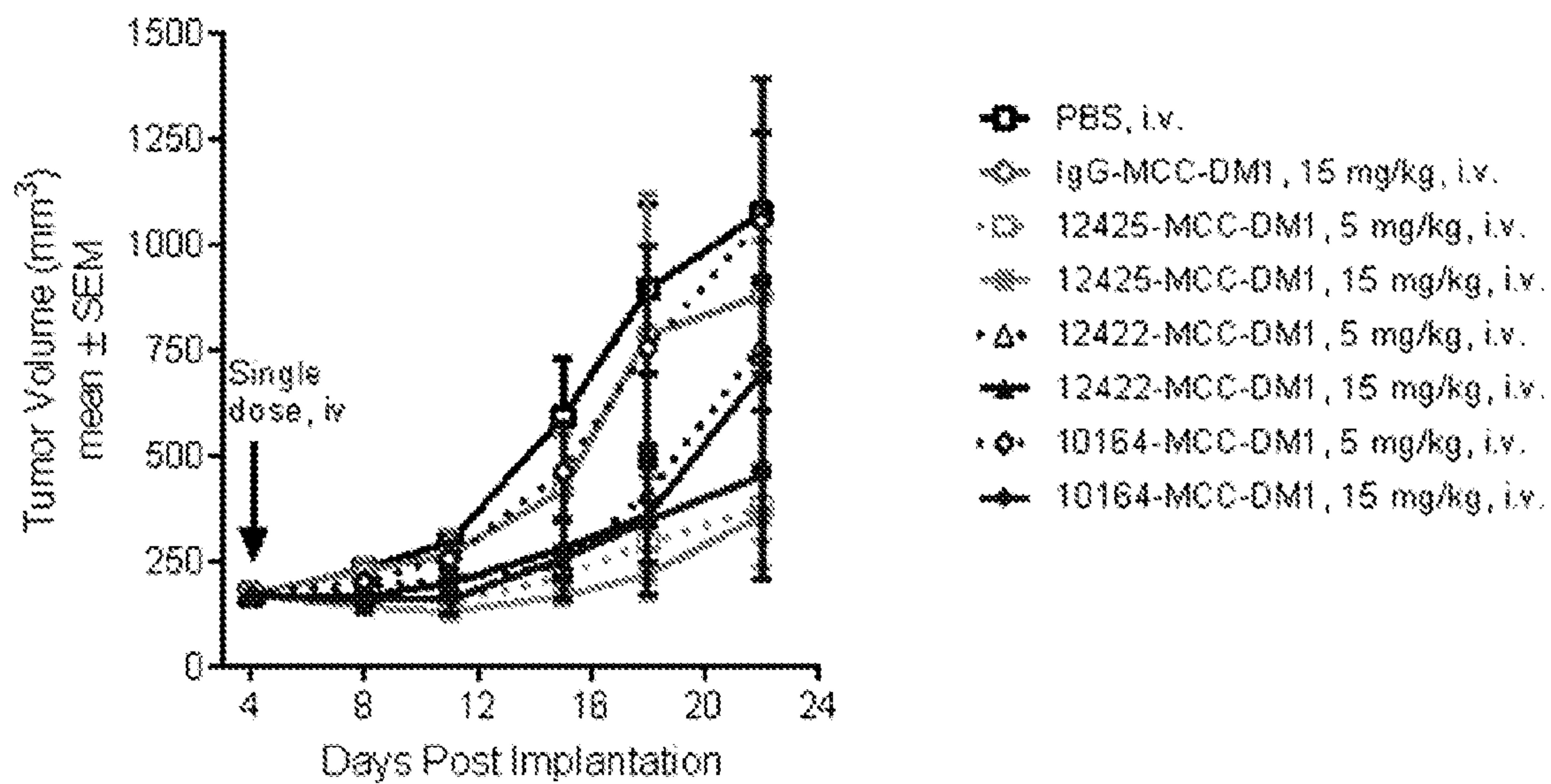


FIG. 8

(A)



(B)

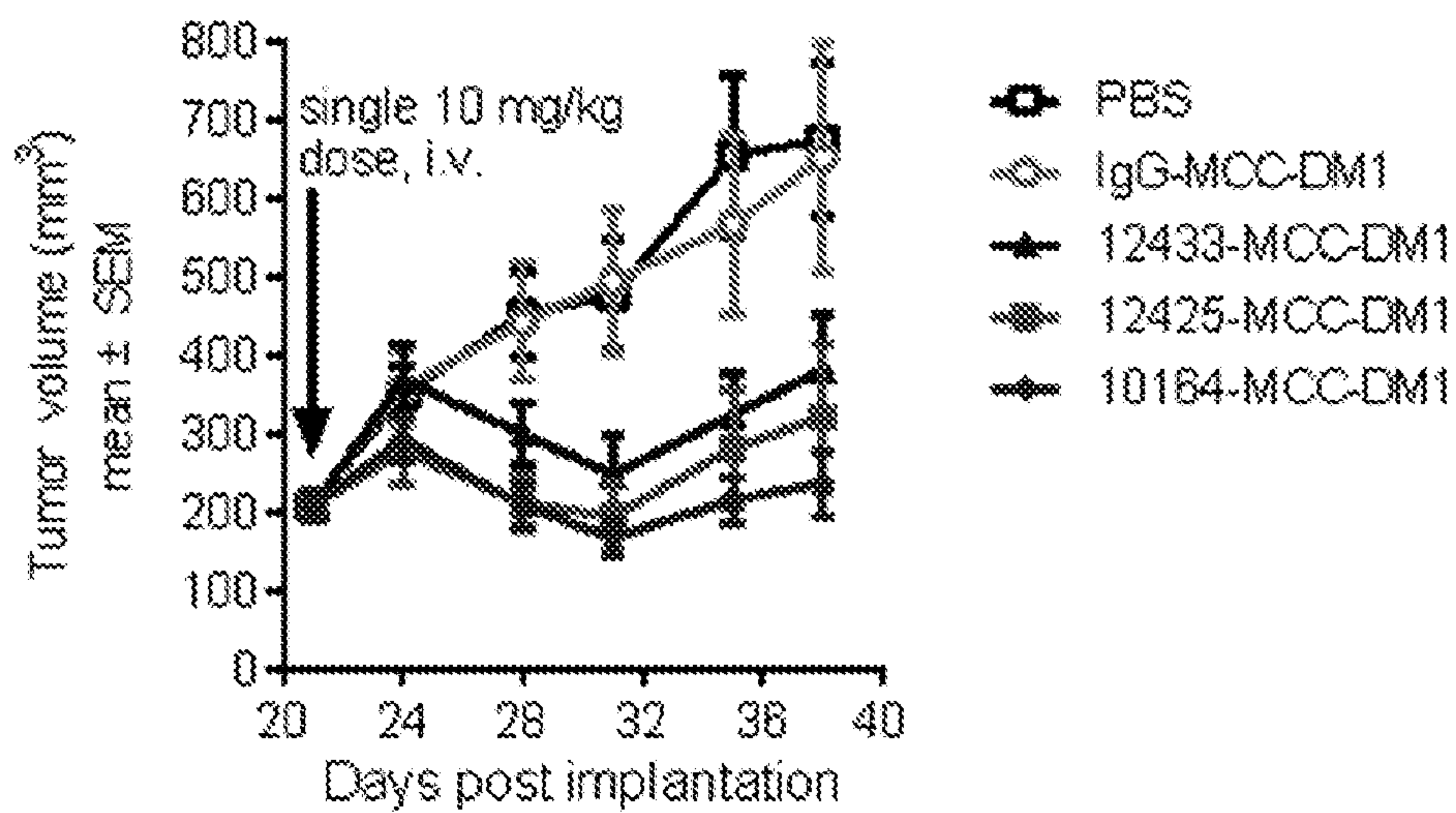
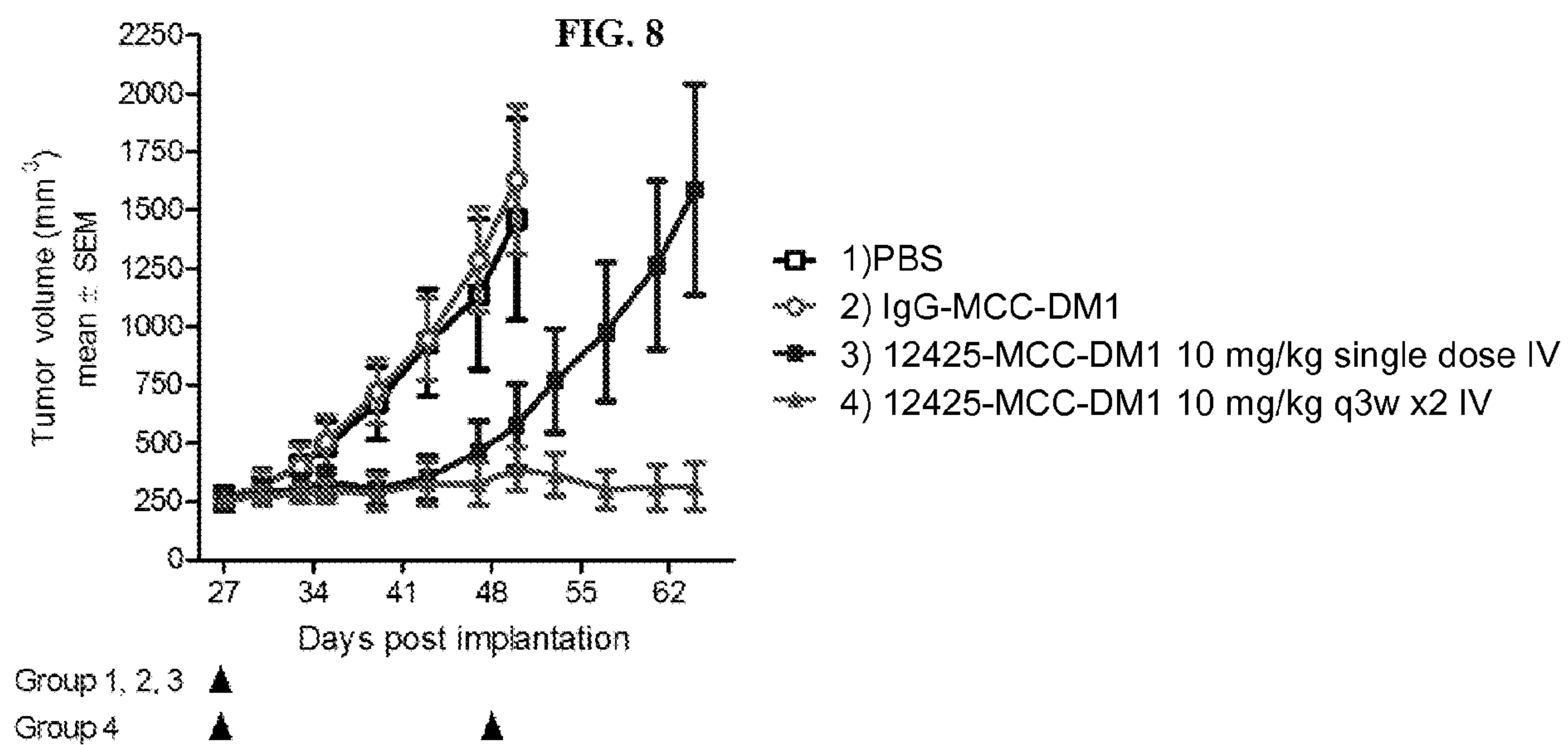


FIG. 8

(C)



(D)

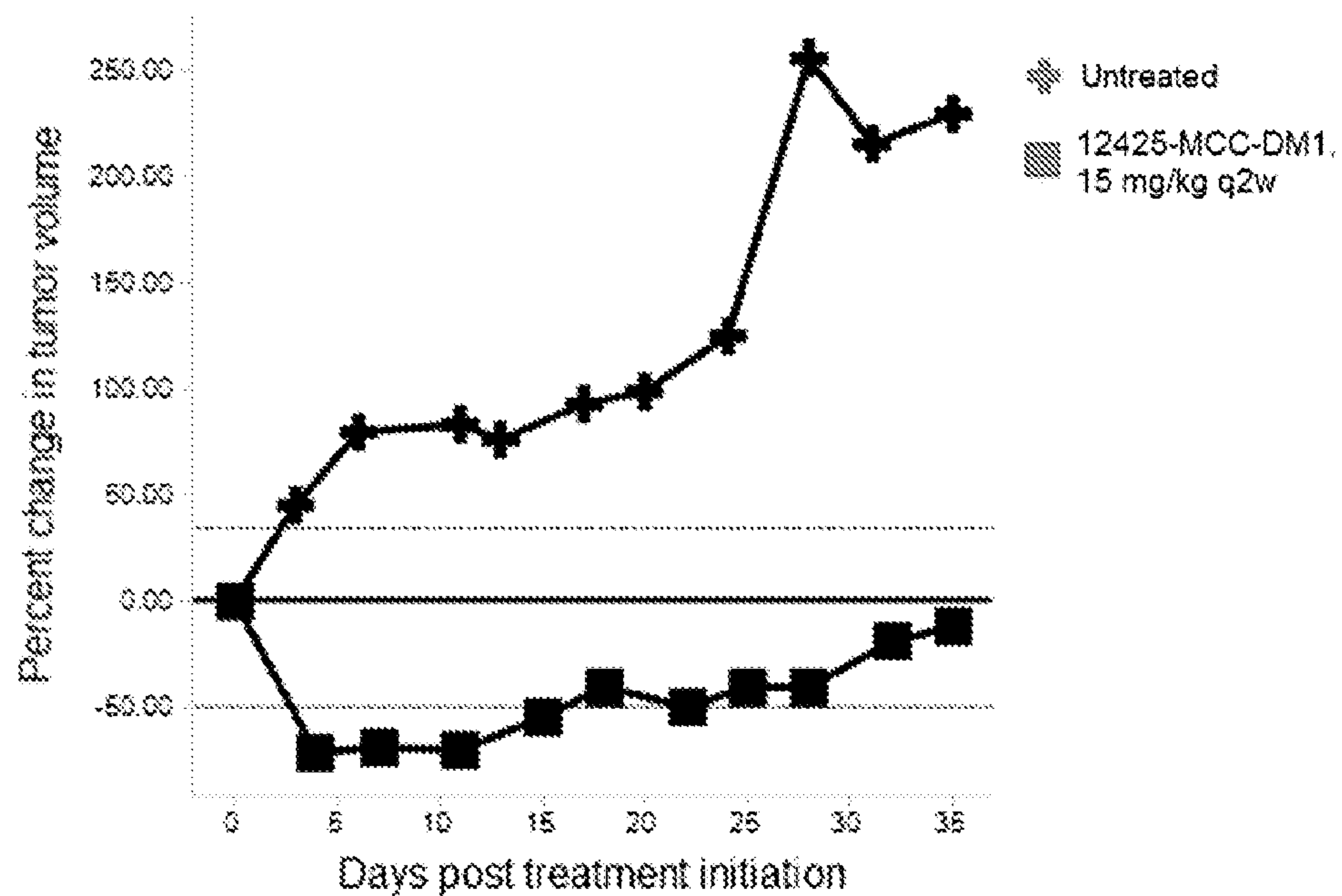
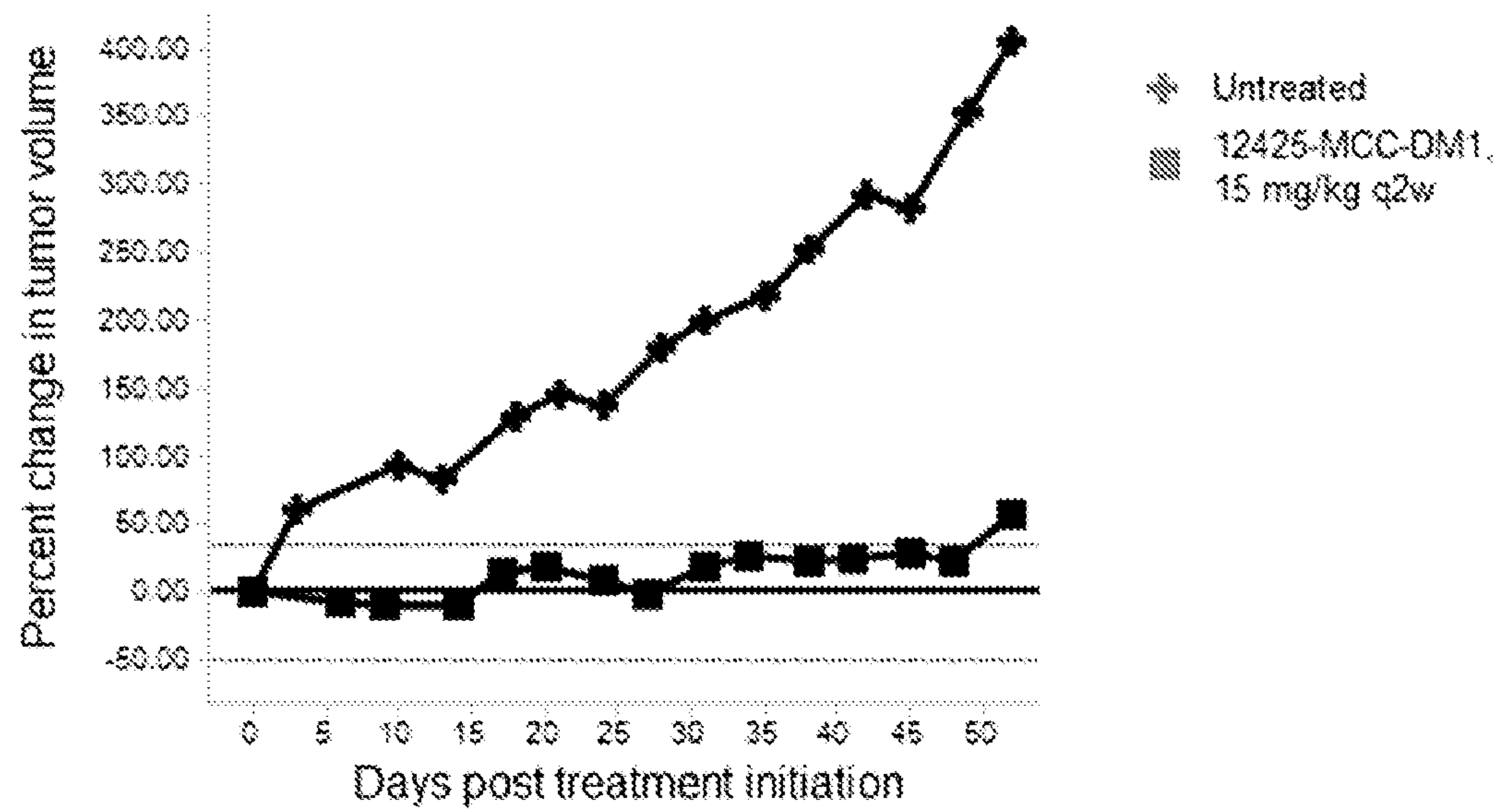


FIG.8

(E)



(F)

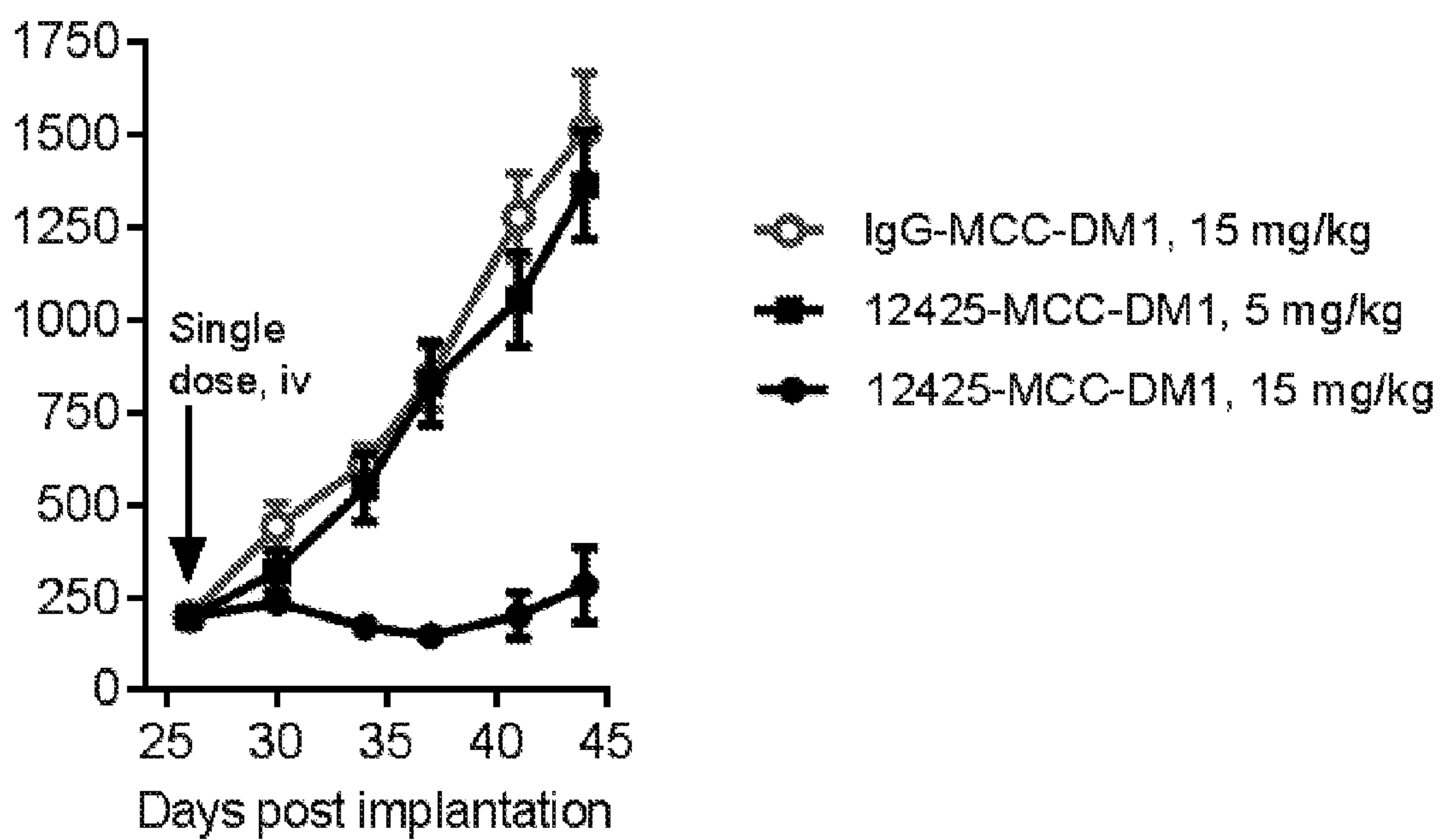


FIG. 9

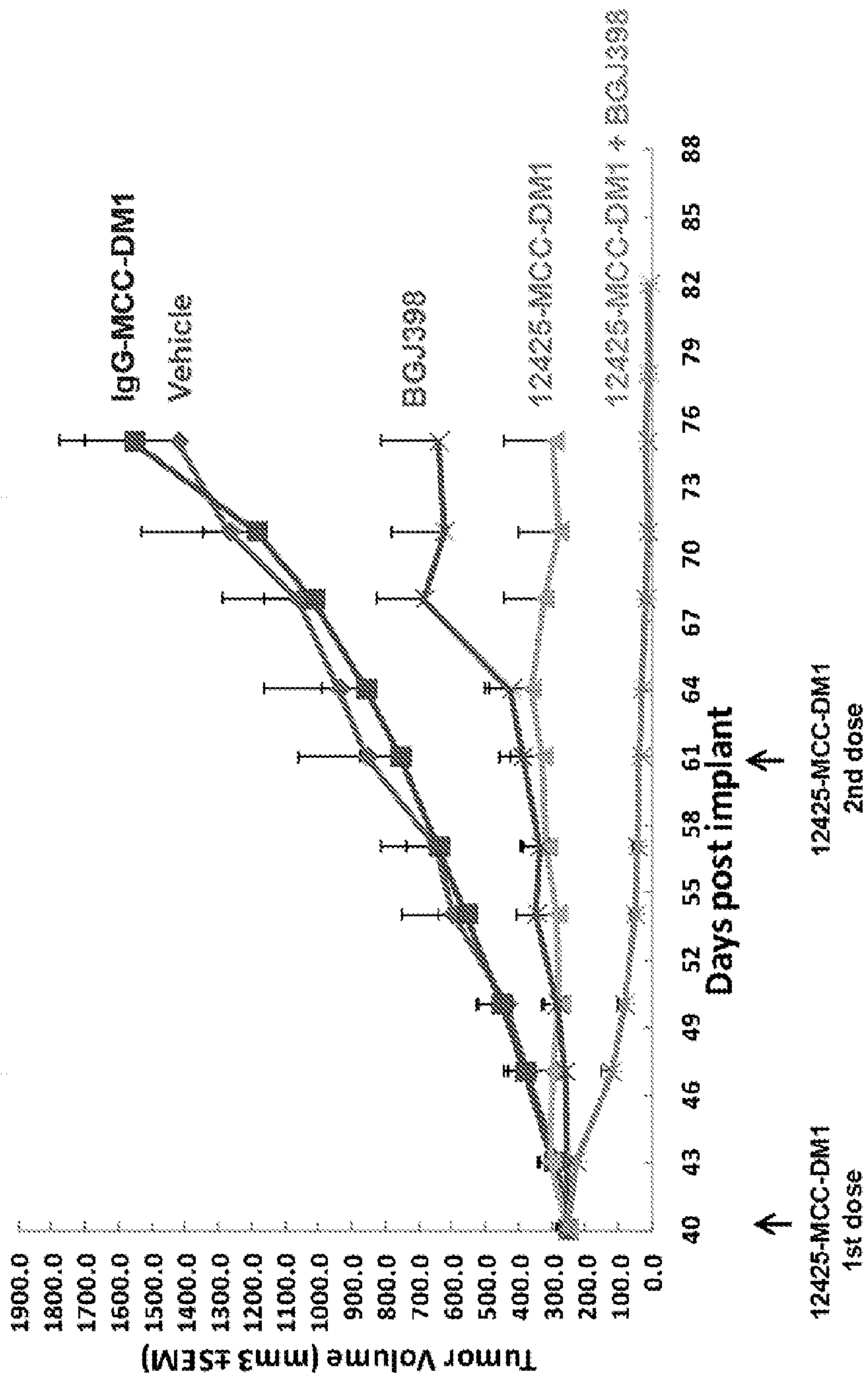


FIG. 10

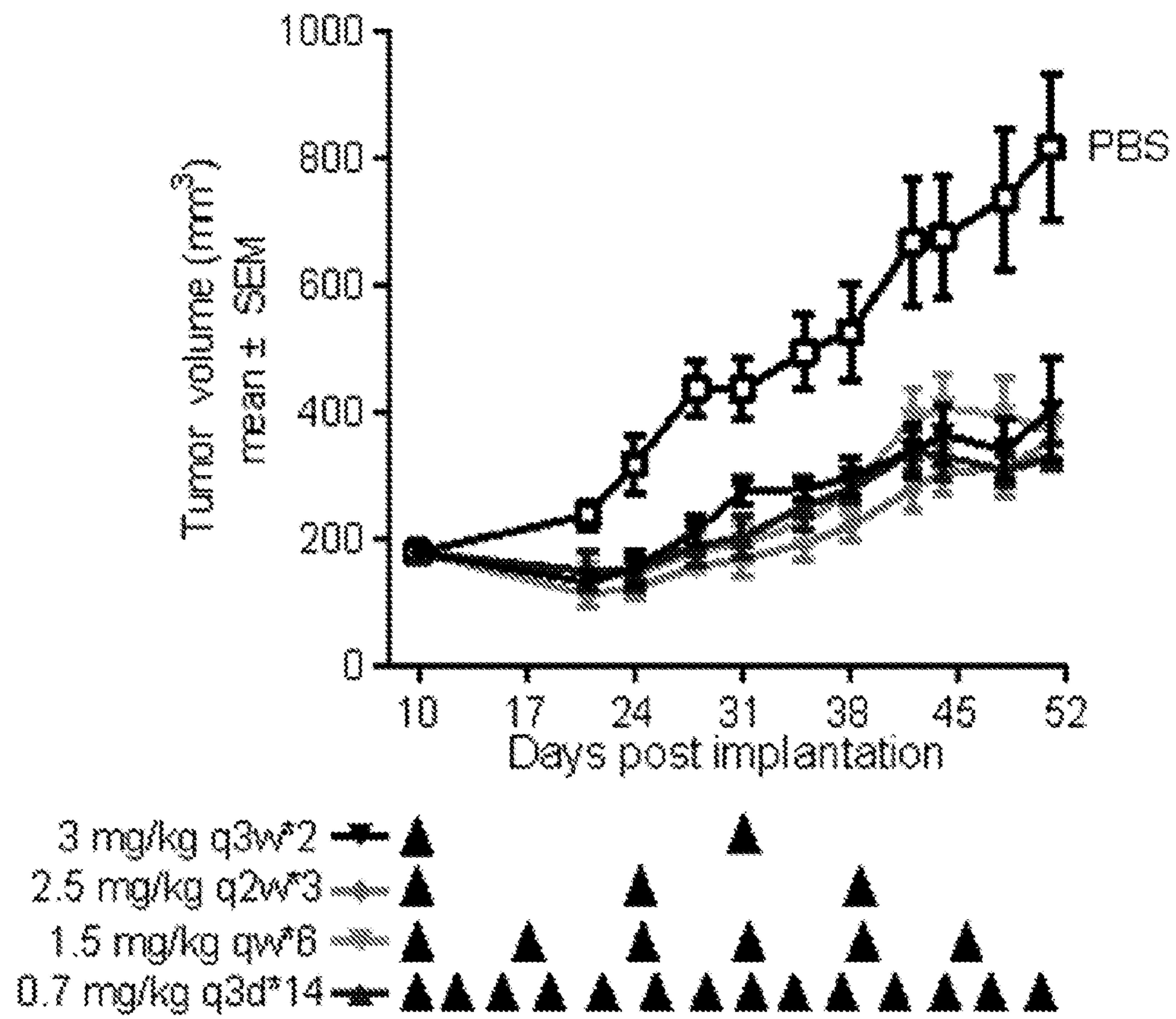
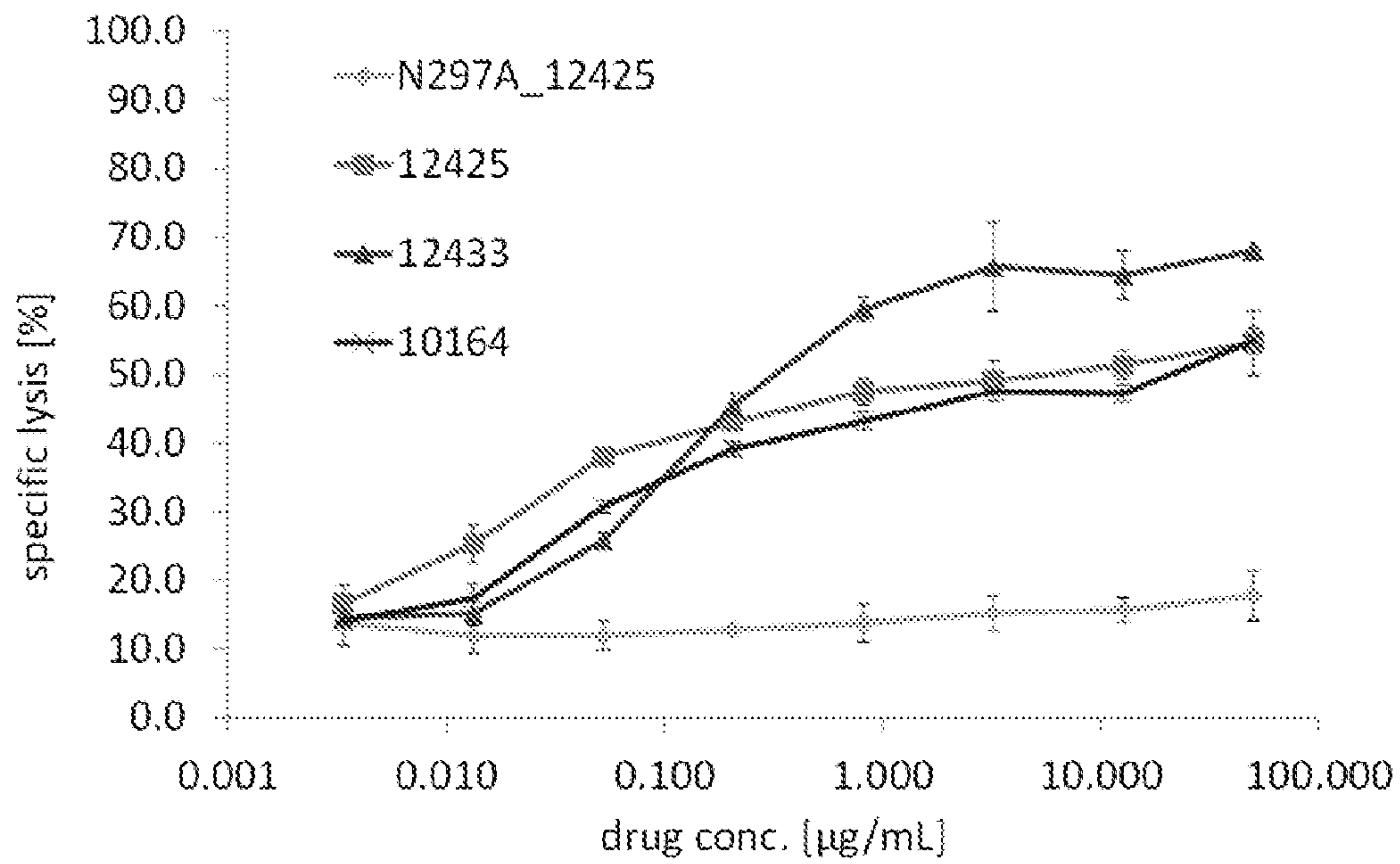


FIG. 11

(A)



(B)

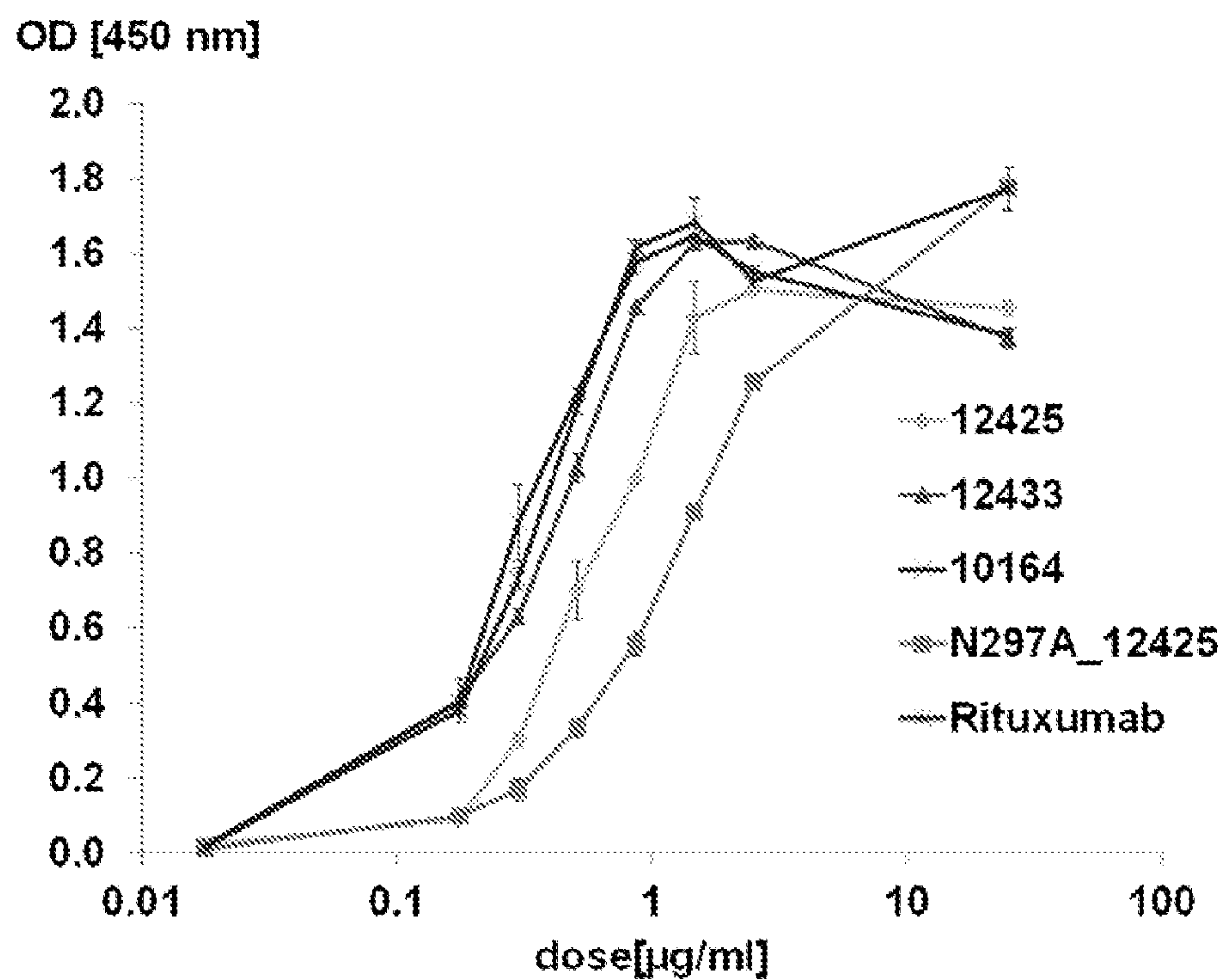


FIG. 11

(C)

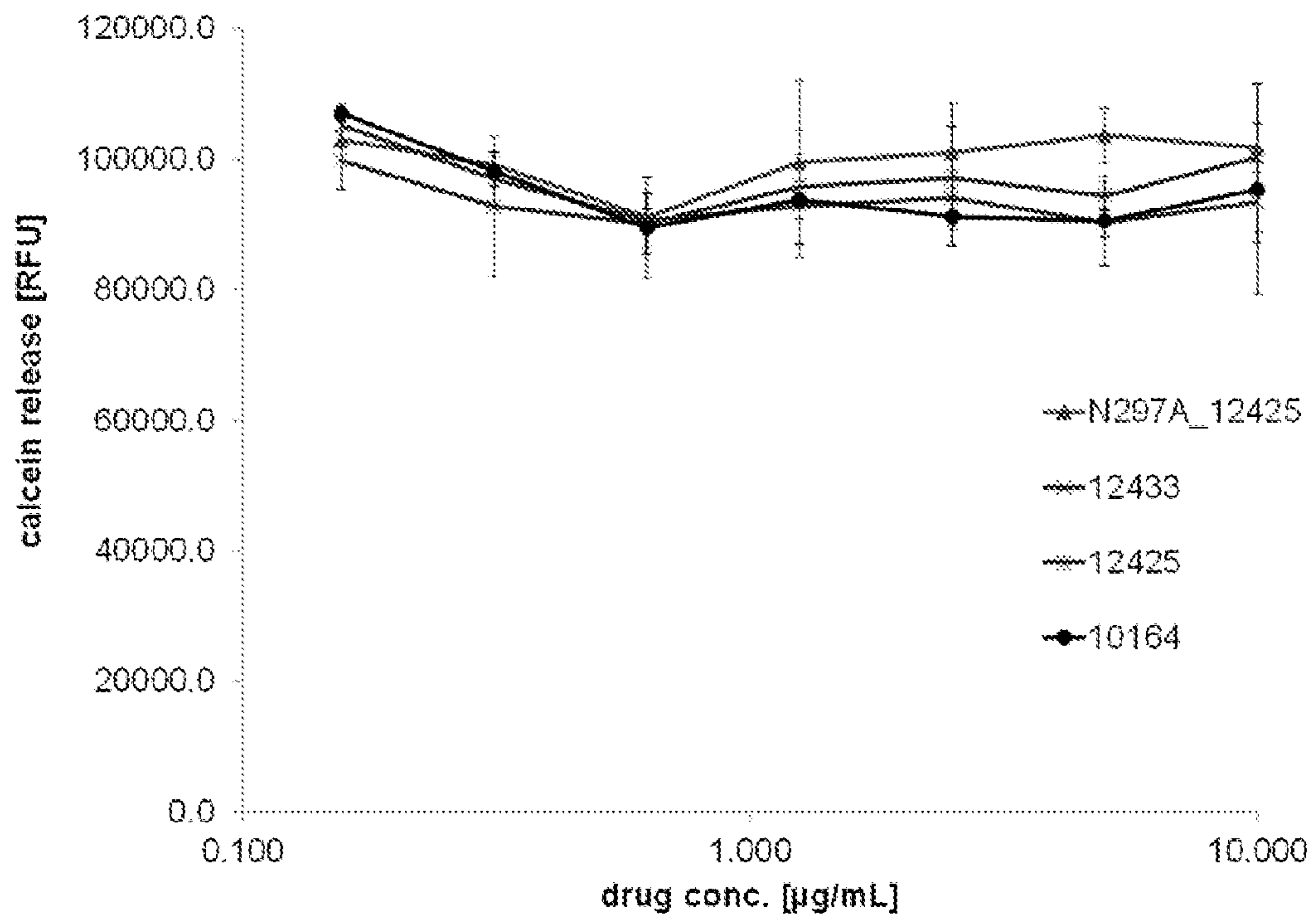


FIG. 11

(D)

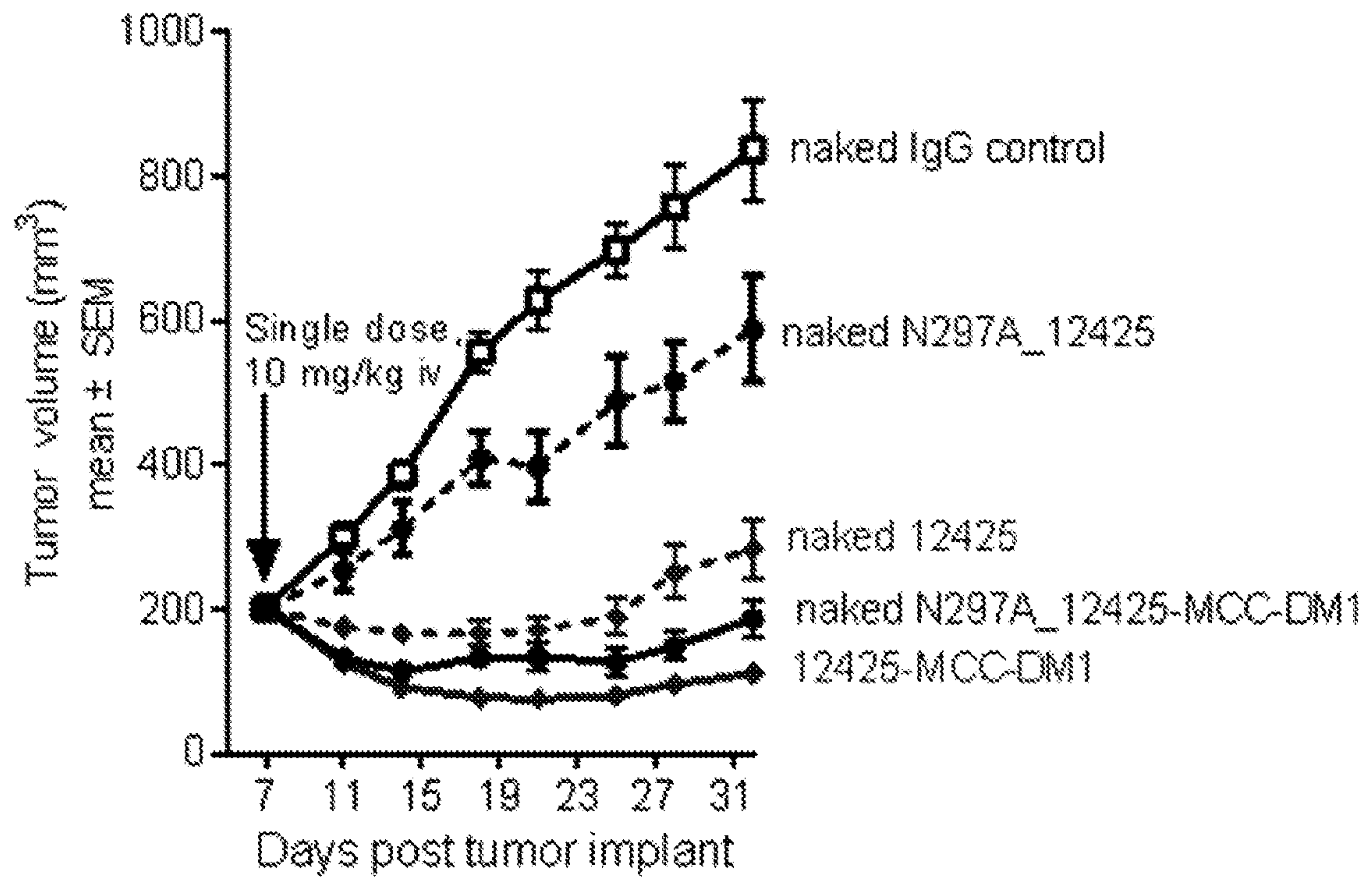
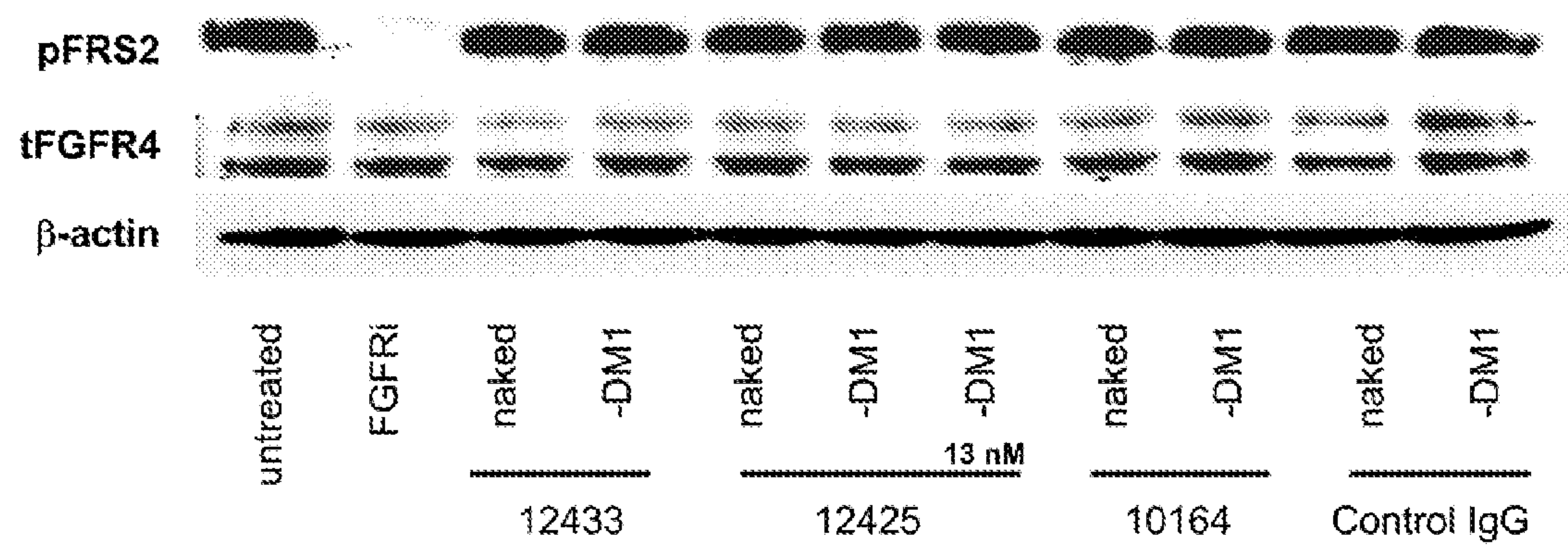


FIG. 12

(A)



(B)

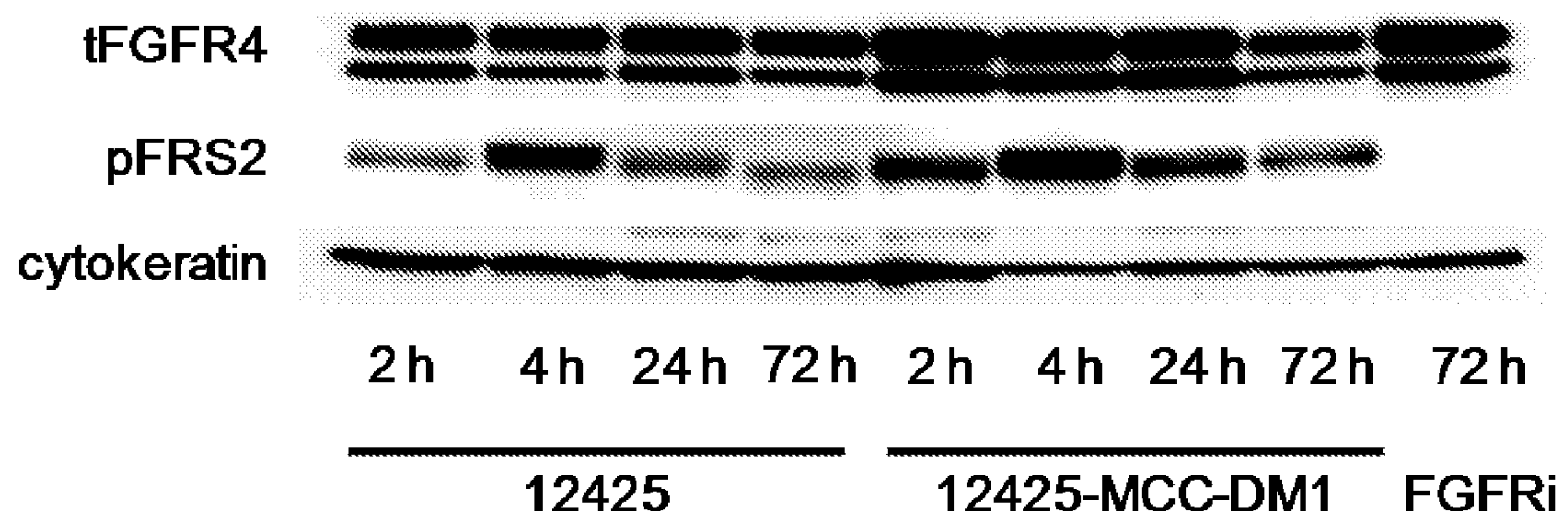
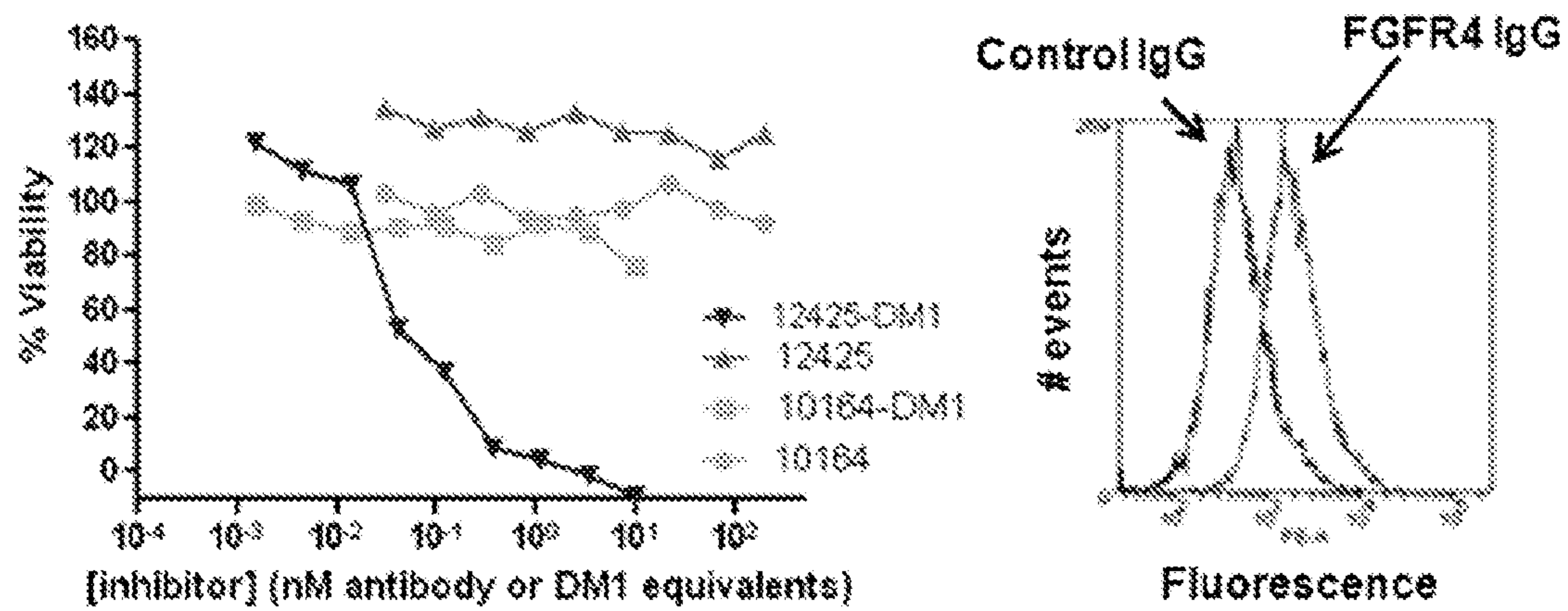


FIG. 13

(A)



(B)

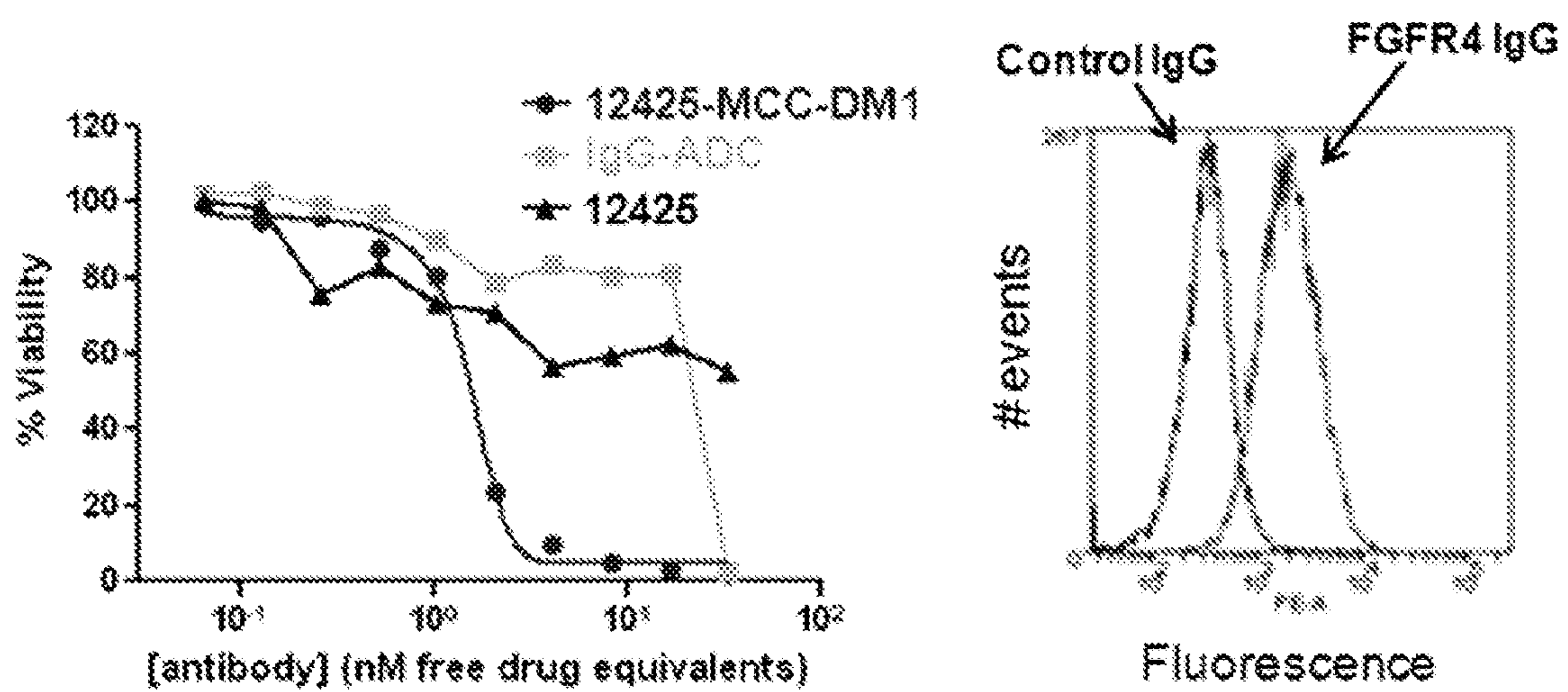


FIG. 13

(C)

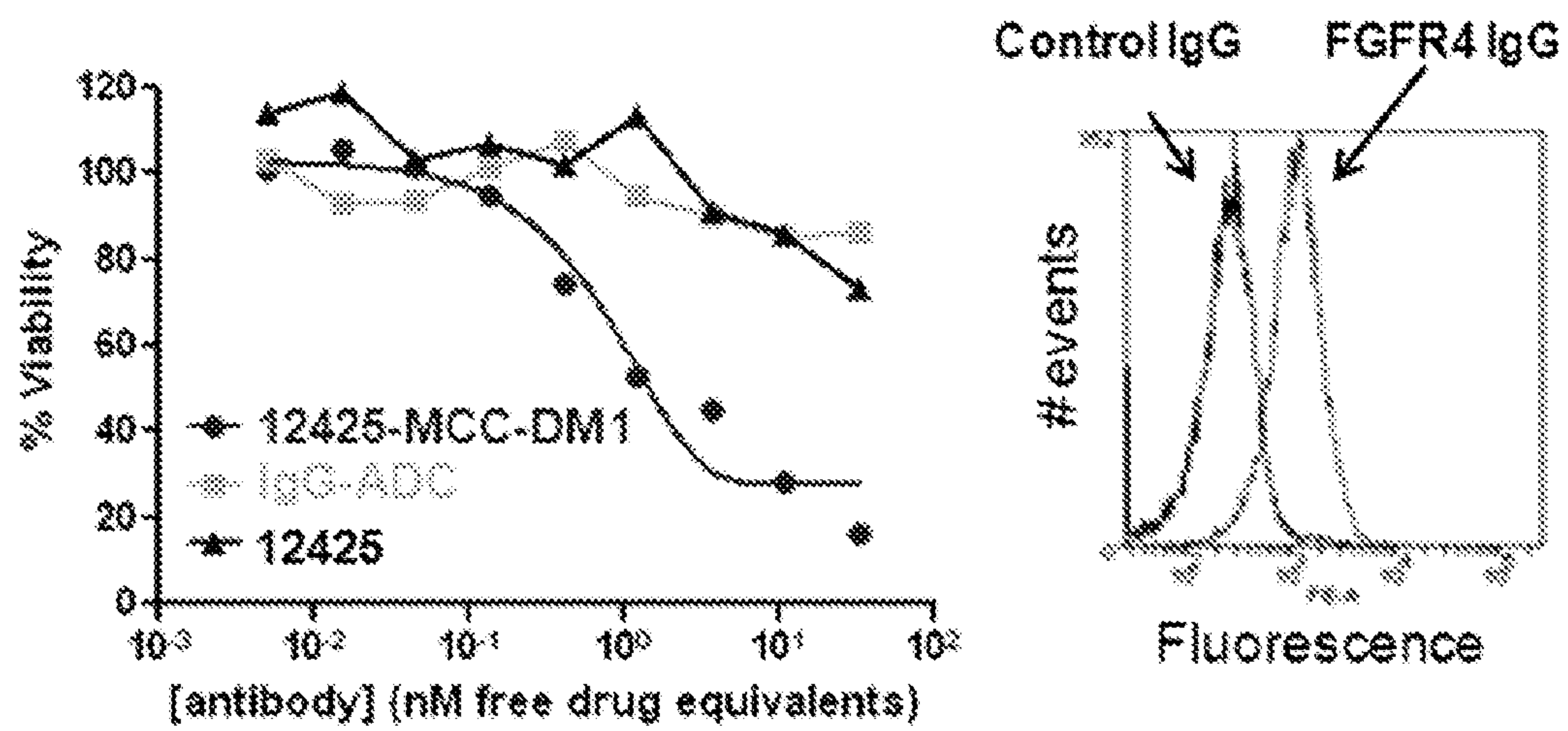


FIG. 13

(D)

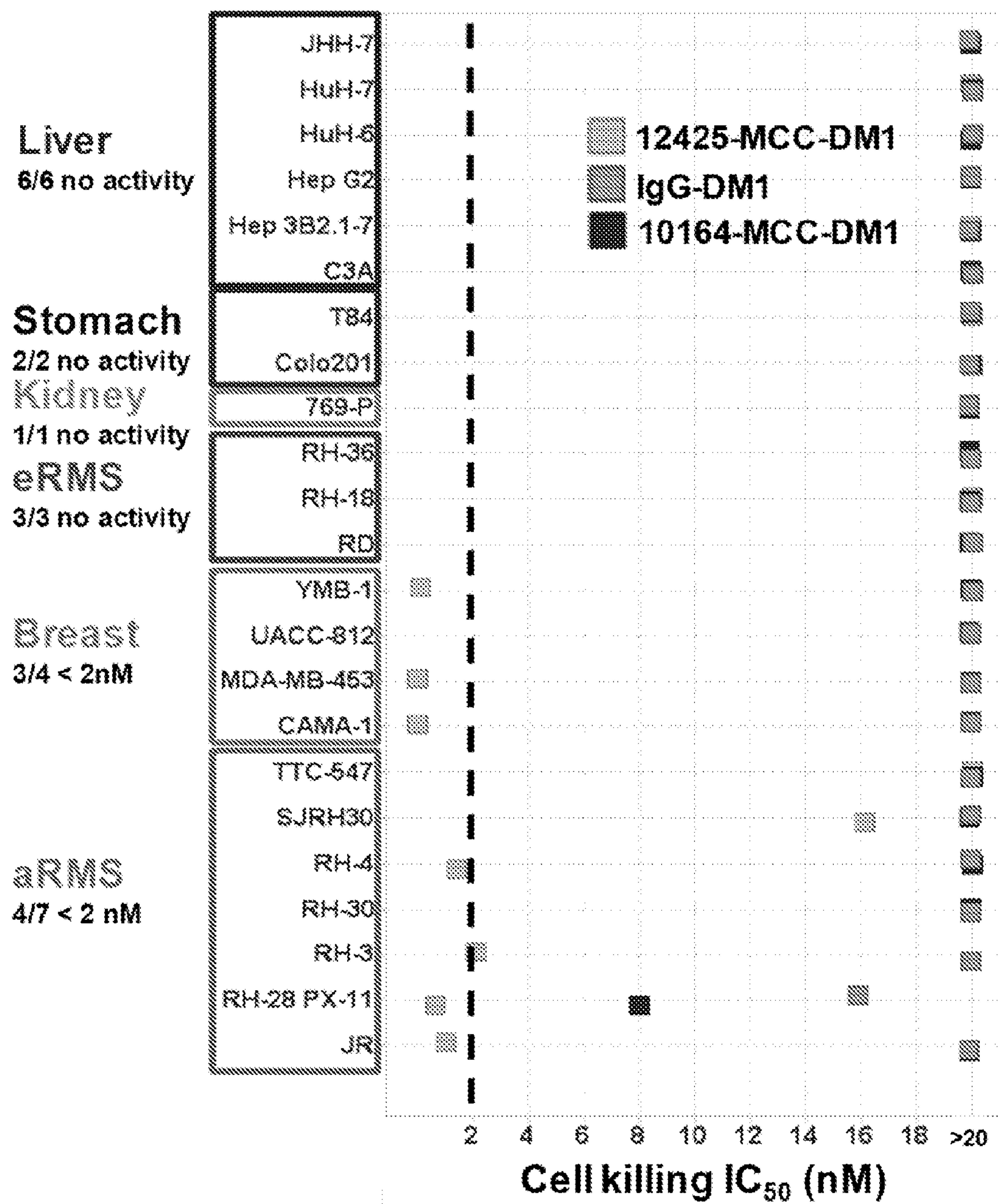
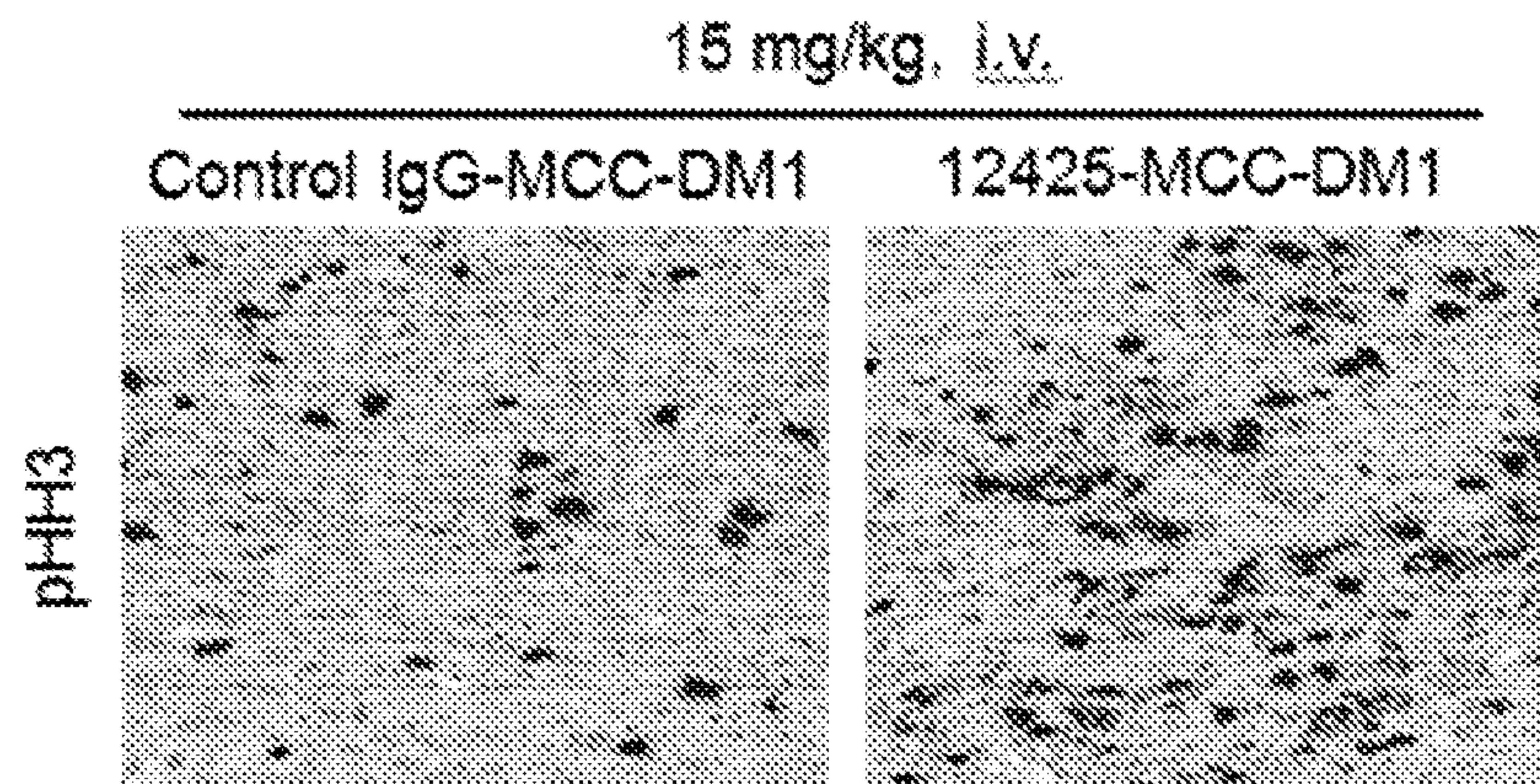
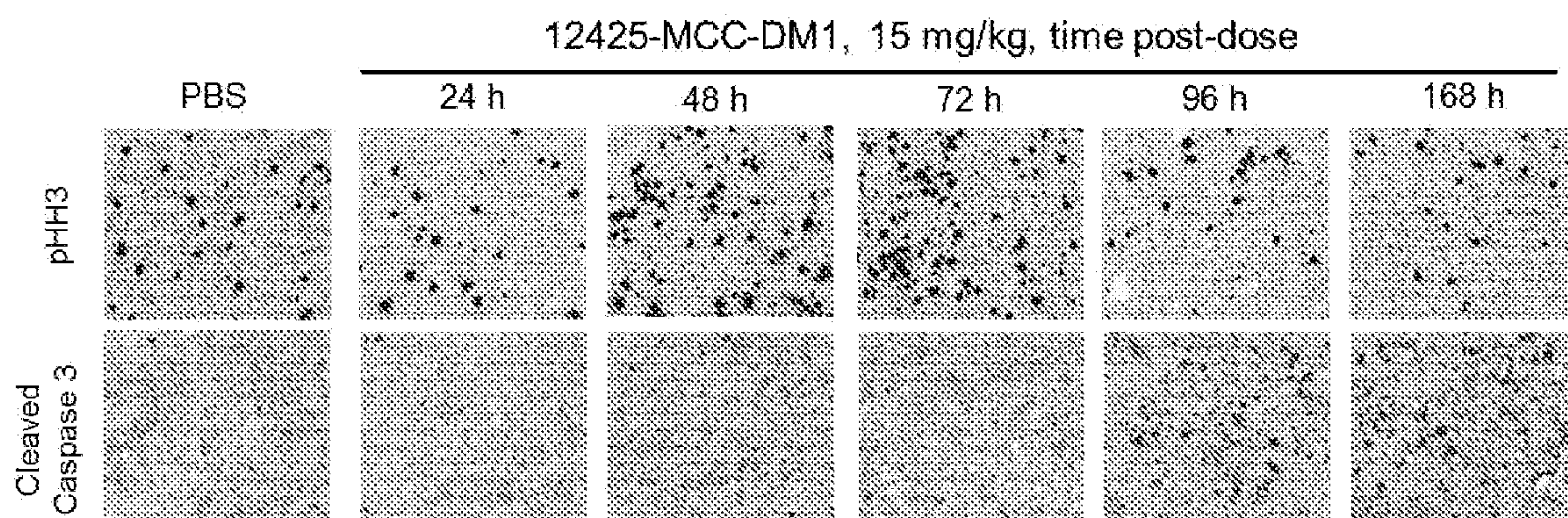


FIG. 14

(A)



(B)



(C)

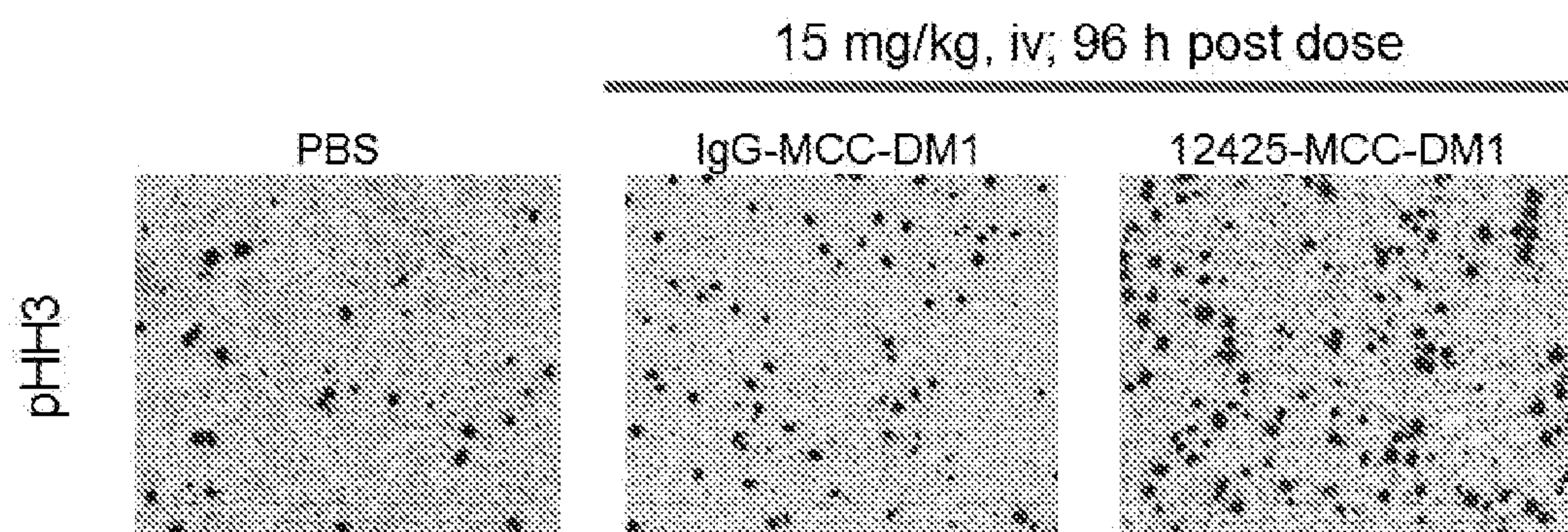
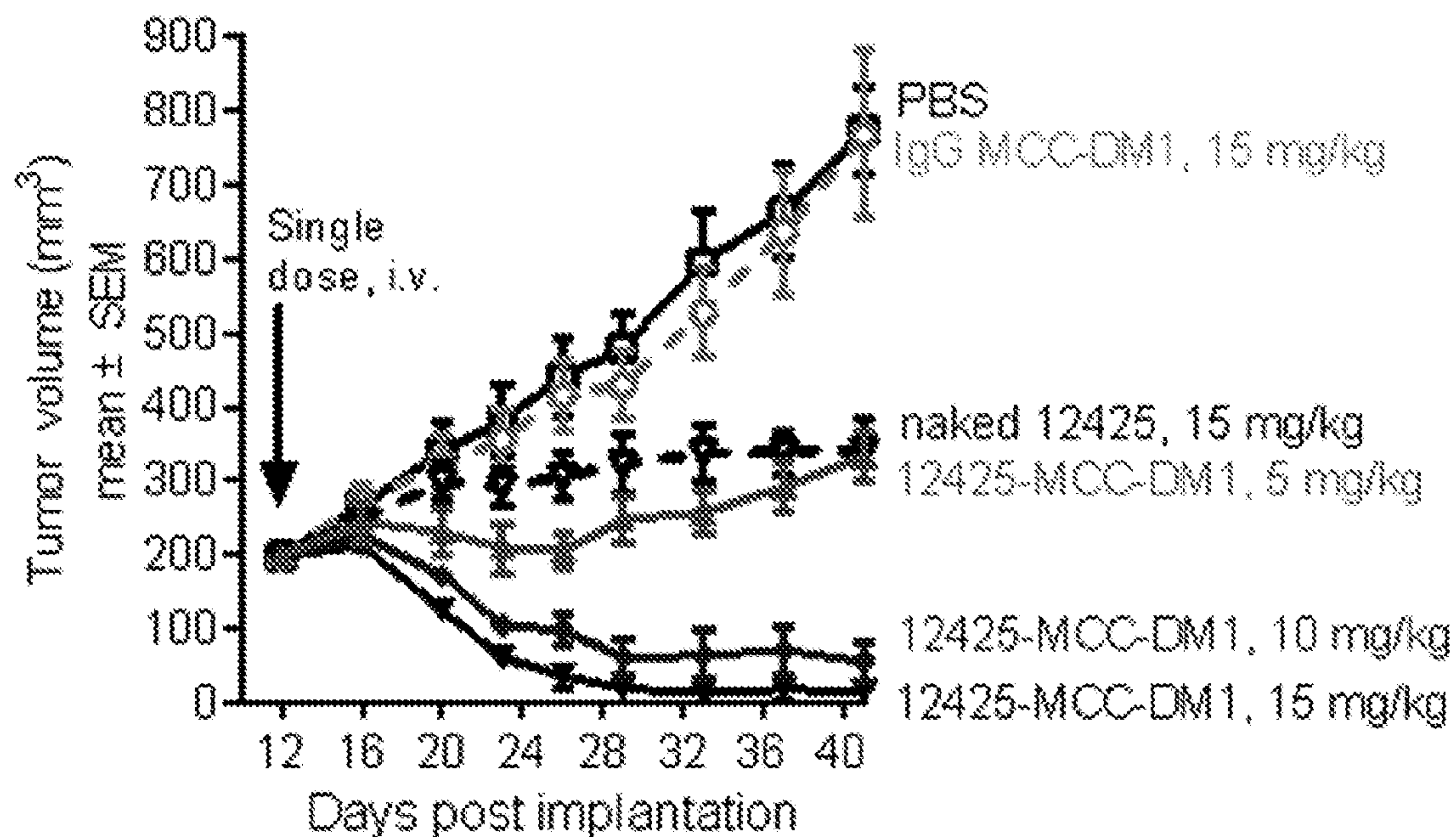


FIG. 15

(A)



(B)

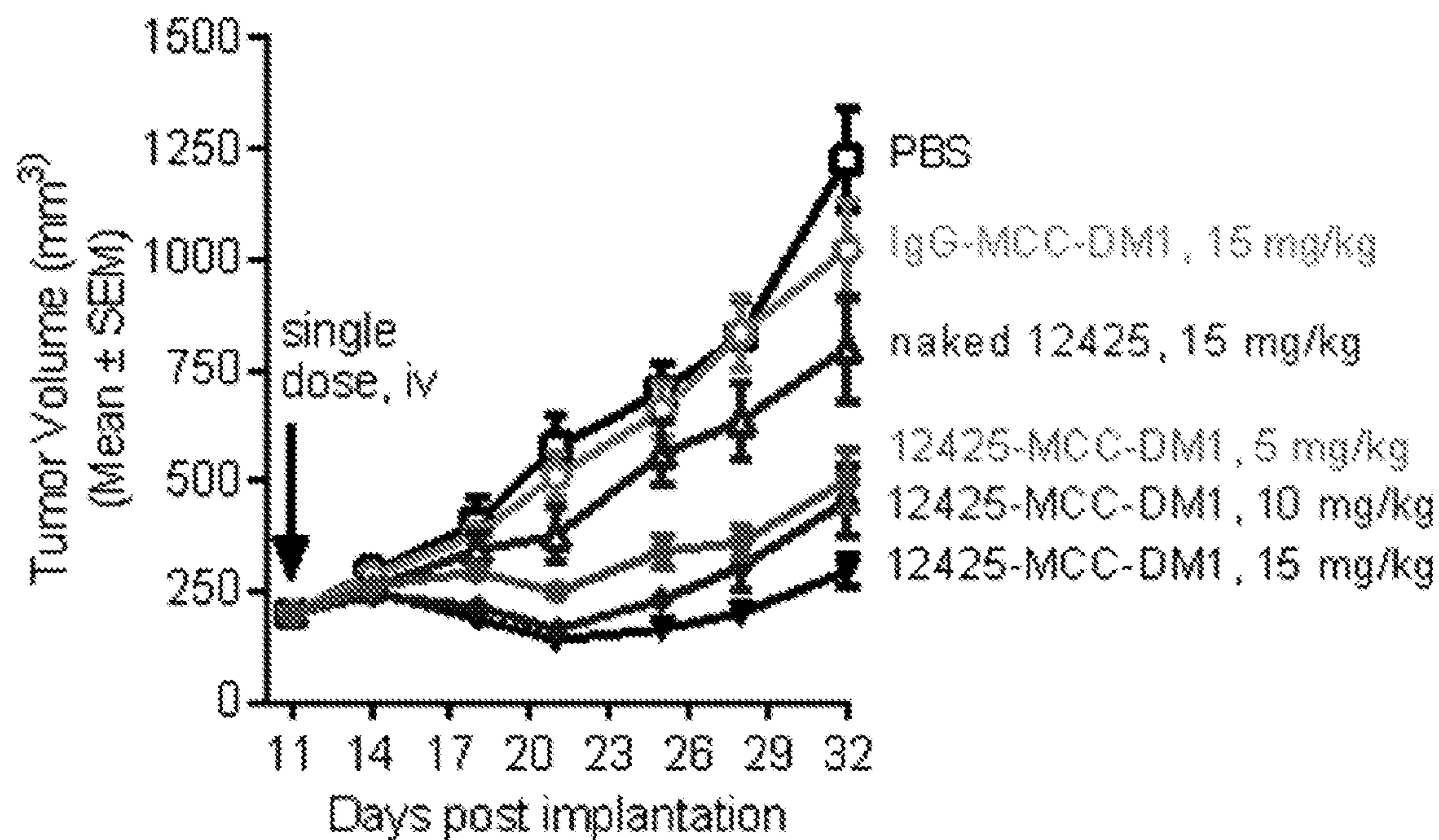
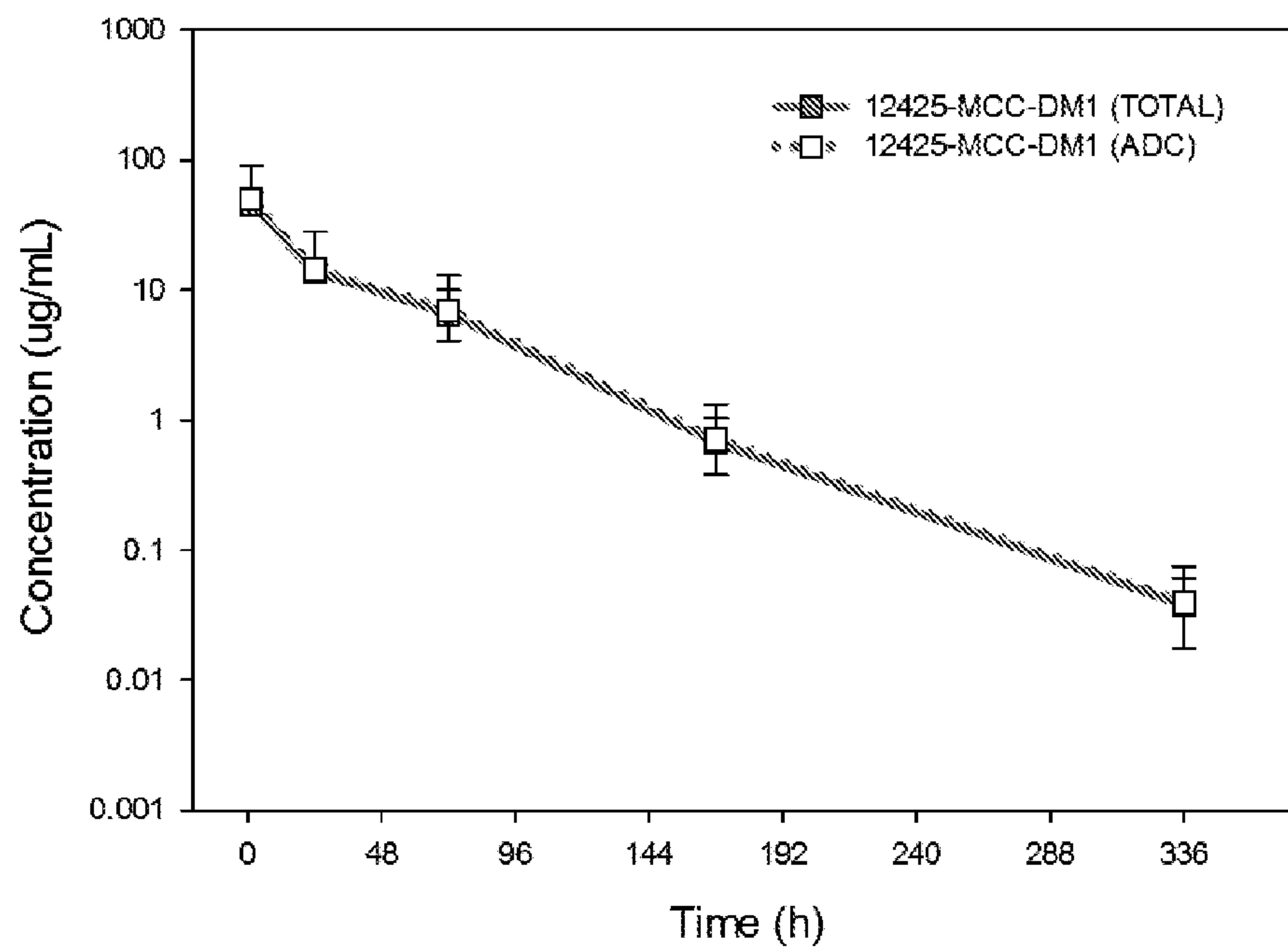


FIG. 16 (A)



(B)

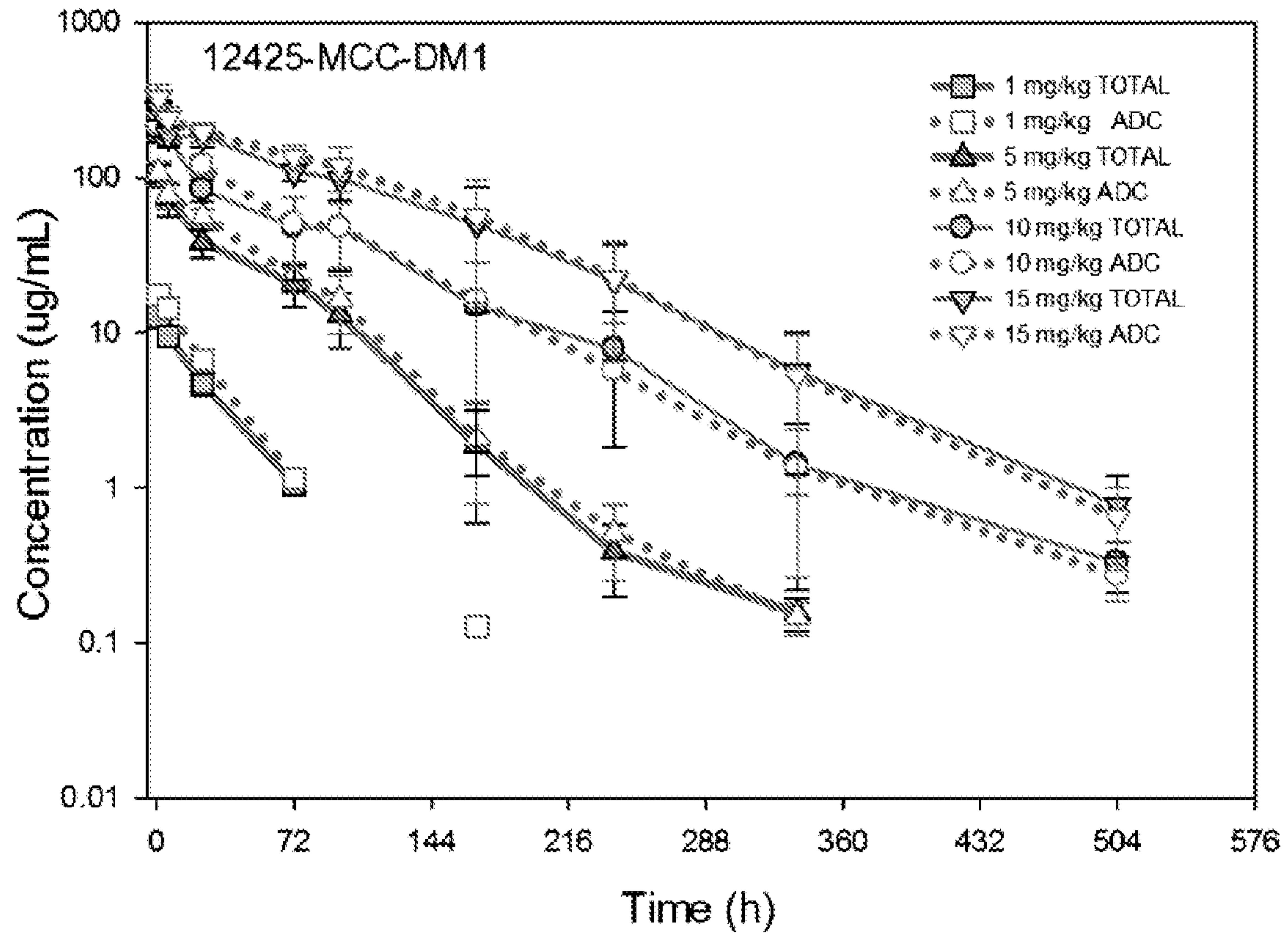
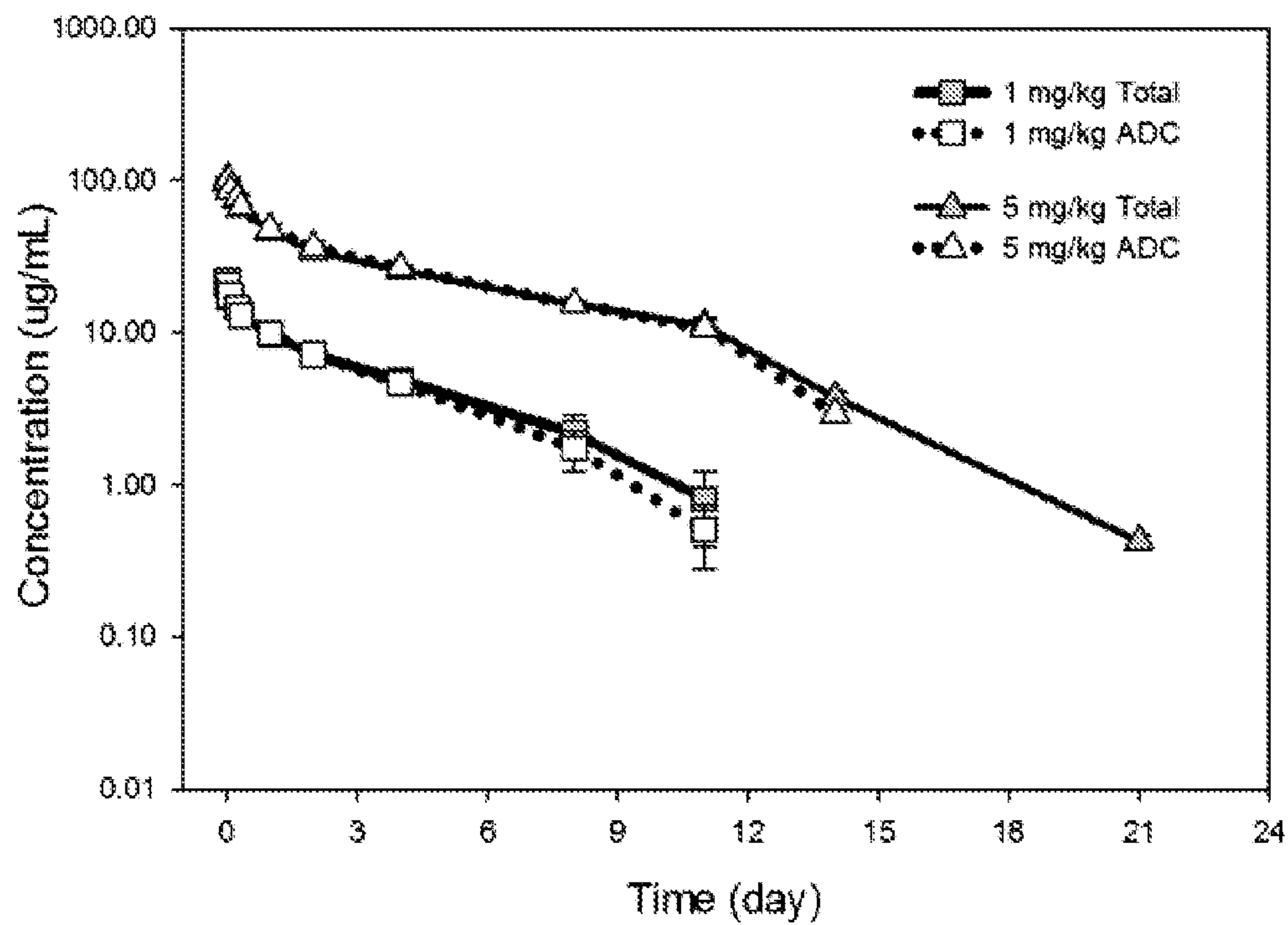


FIG. 16(C)



(D)

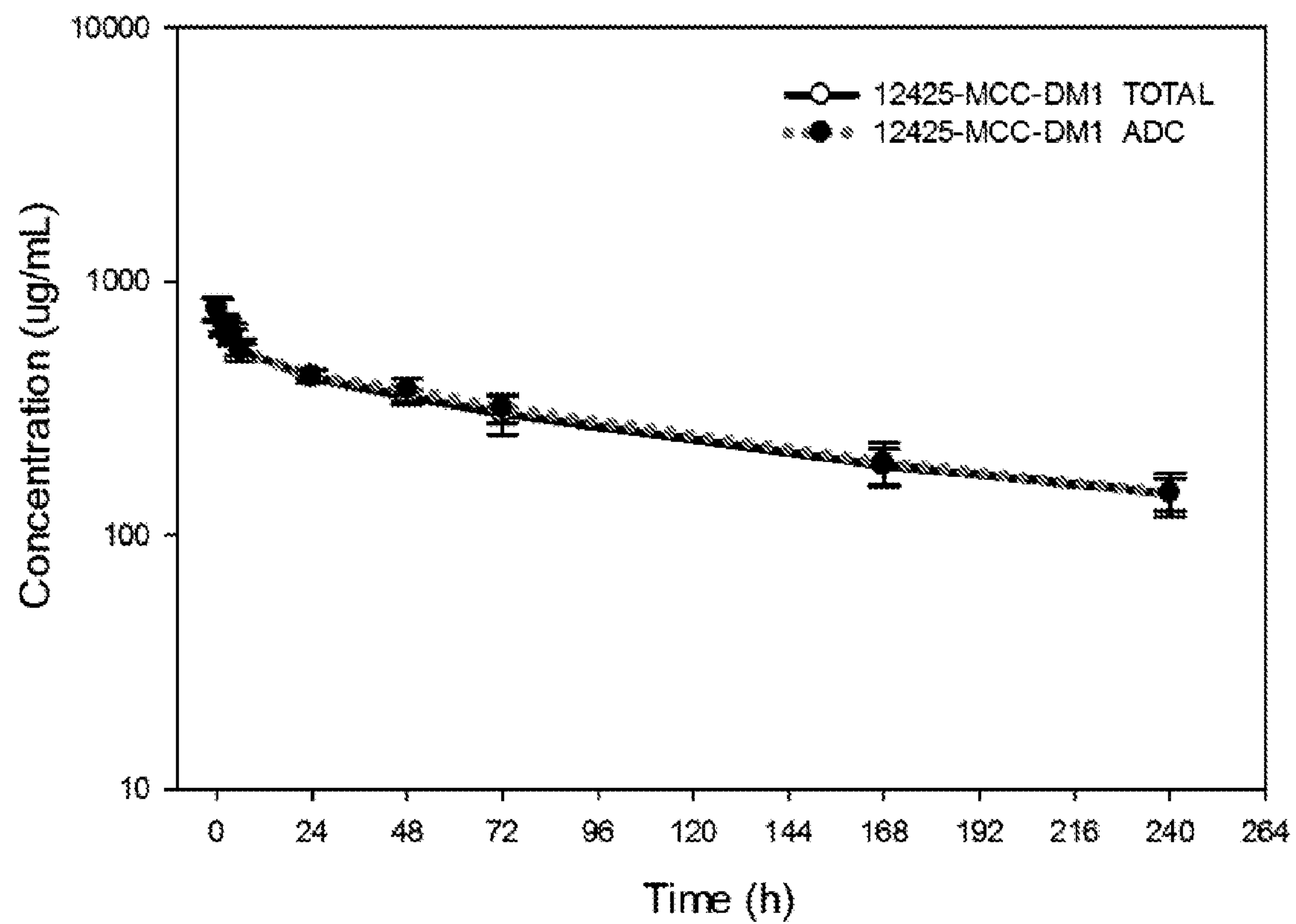
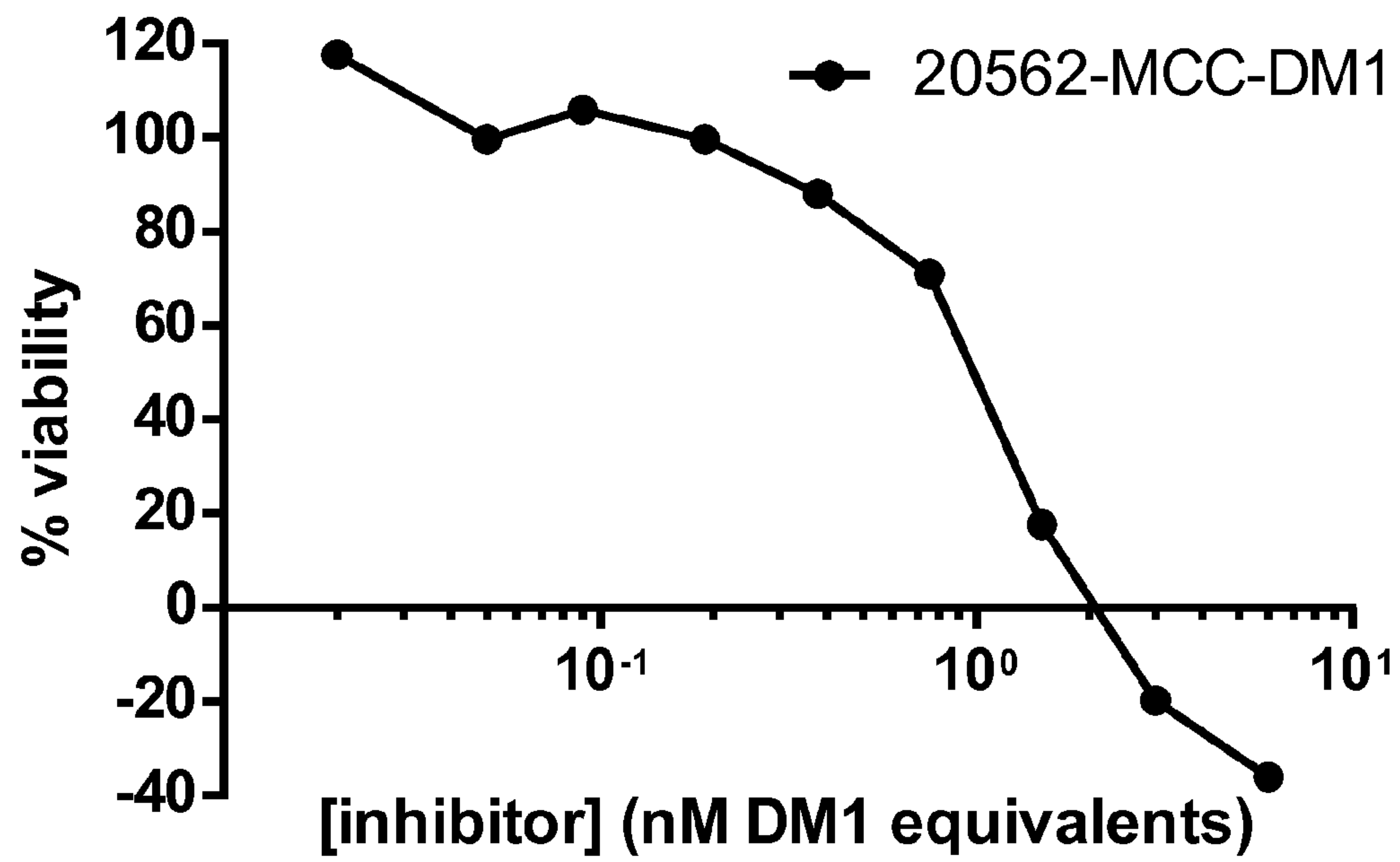


FIG. 17

(A)



(B)

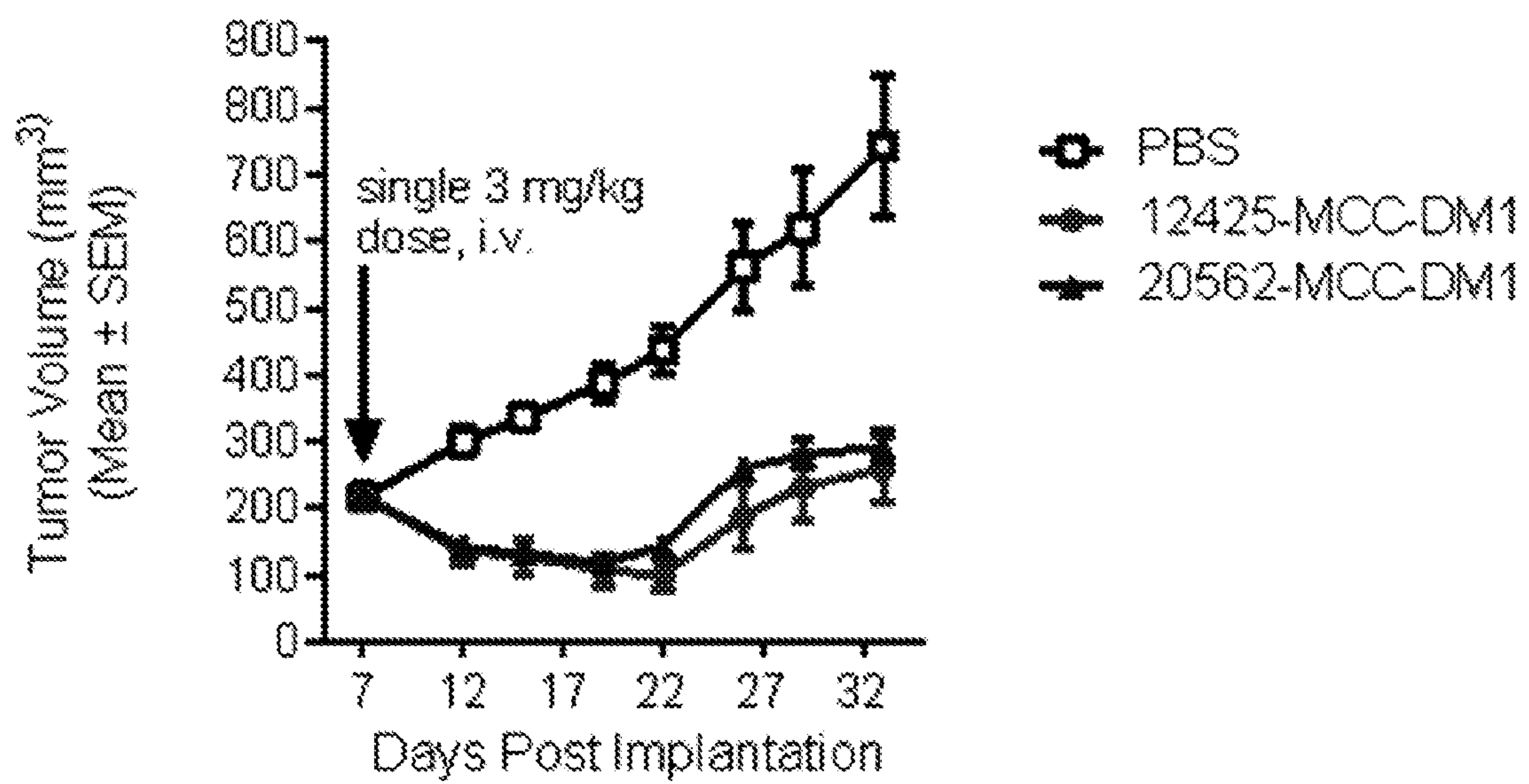
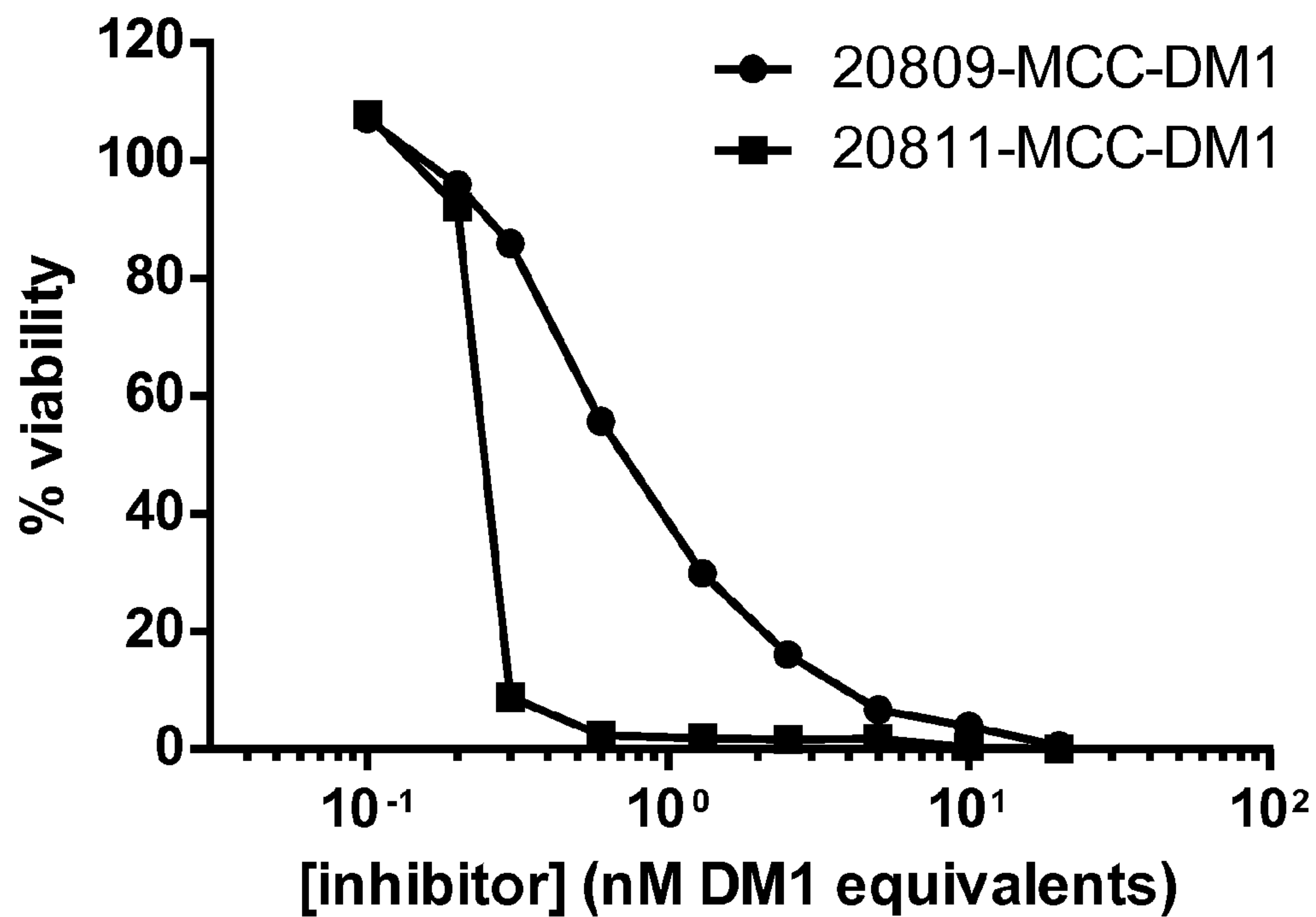


FIG. 18

(A)



(B)

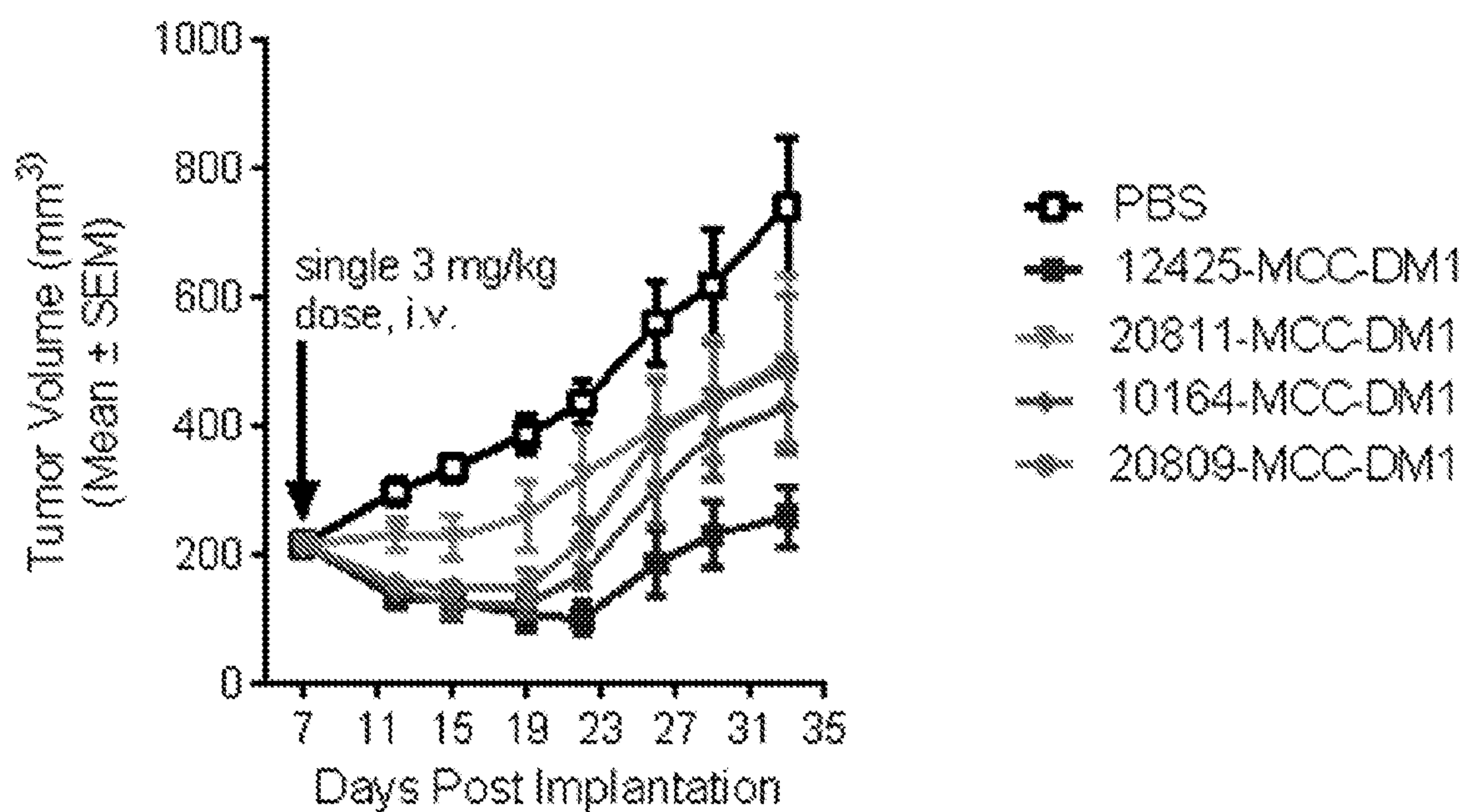


FIG. 19 (A)

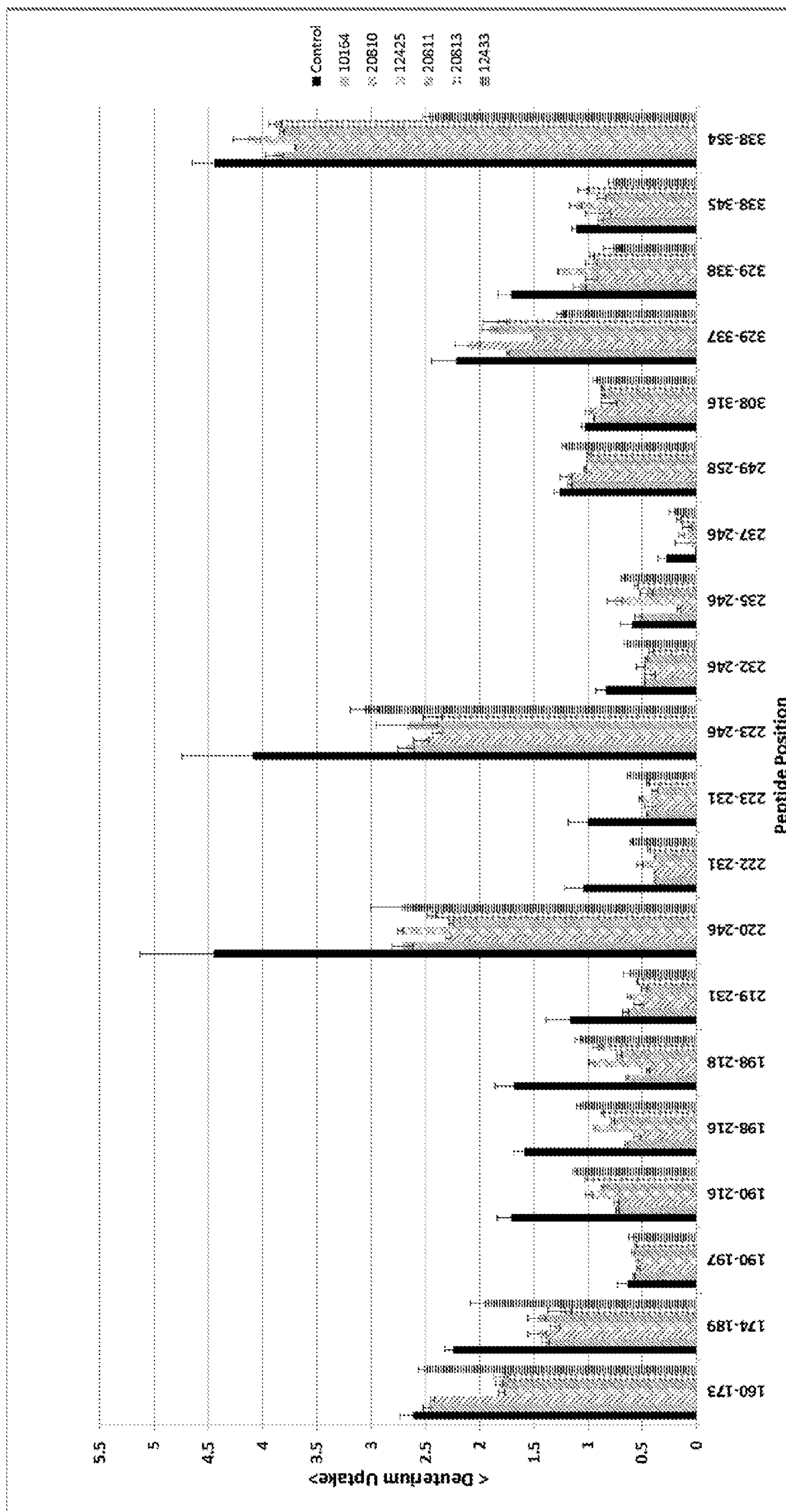


FIG. 19 (B)

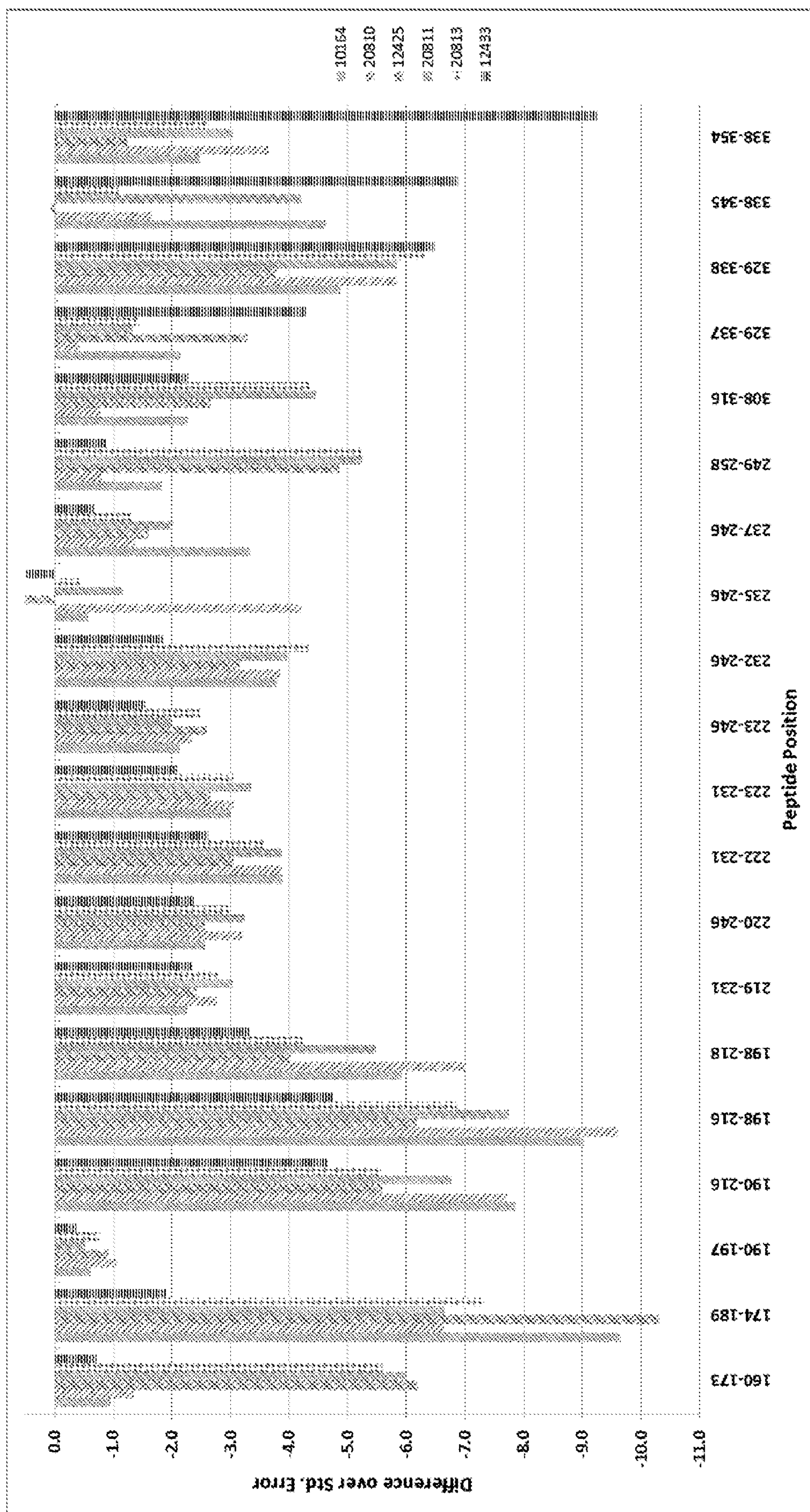


FIG. 19 (C)

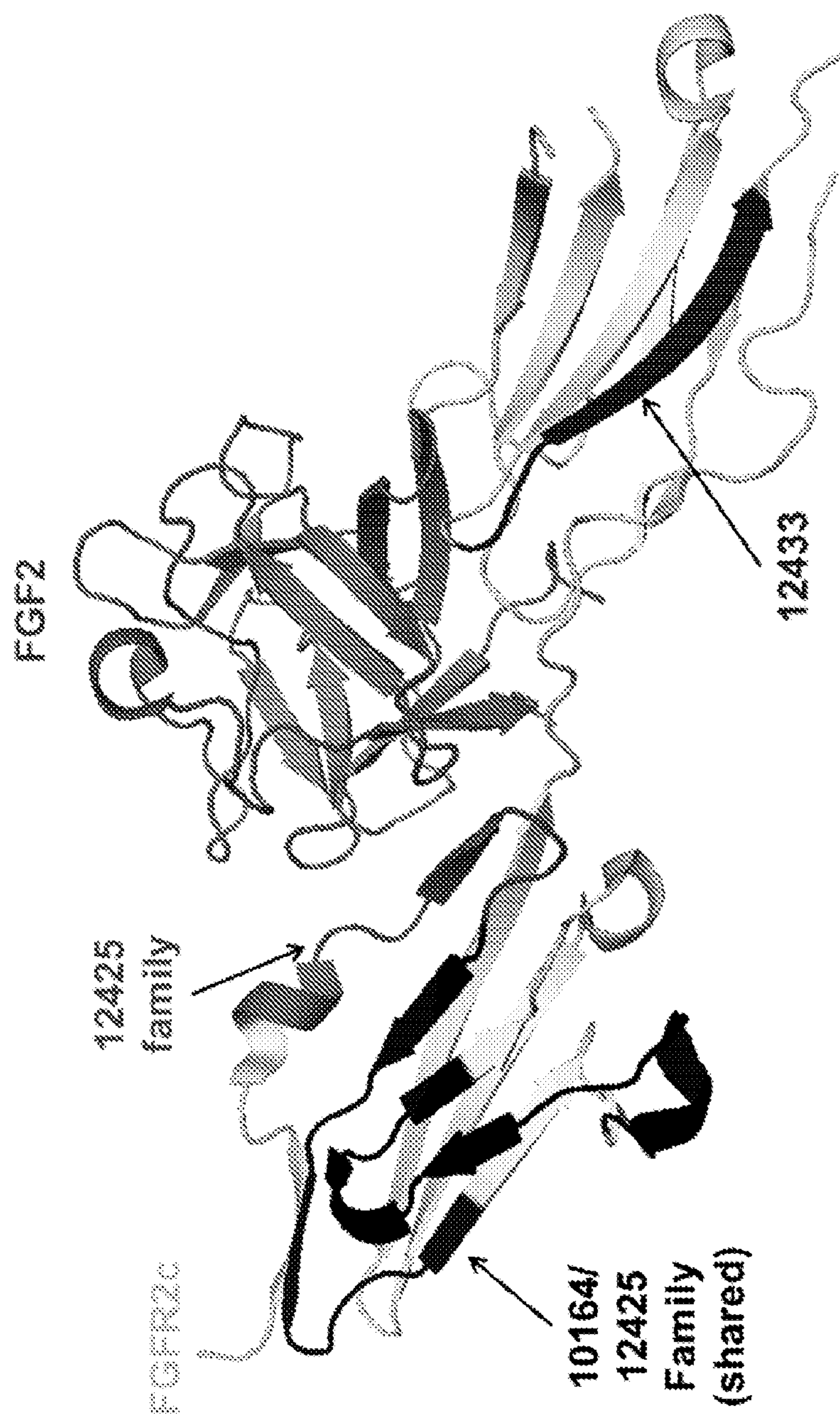


FIG. 19 (D)

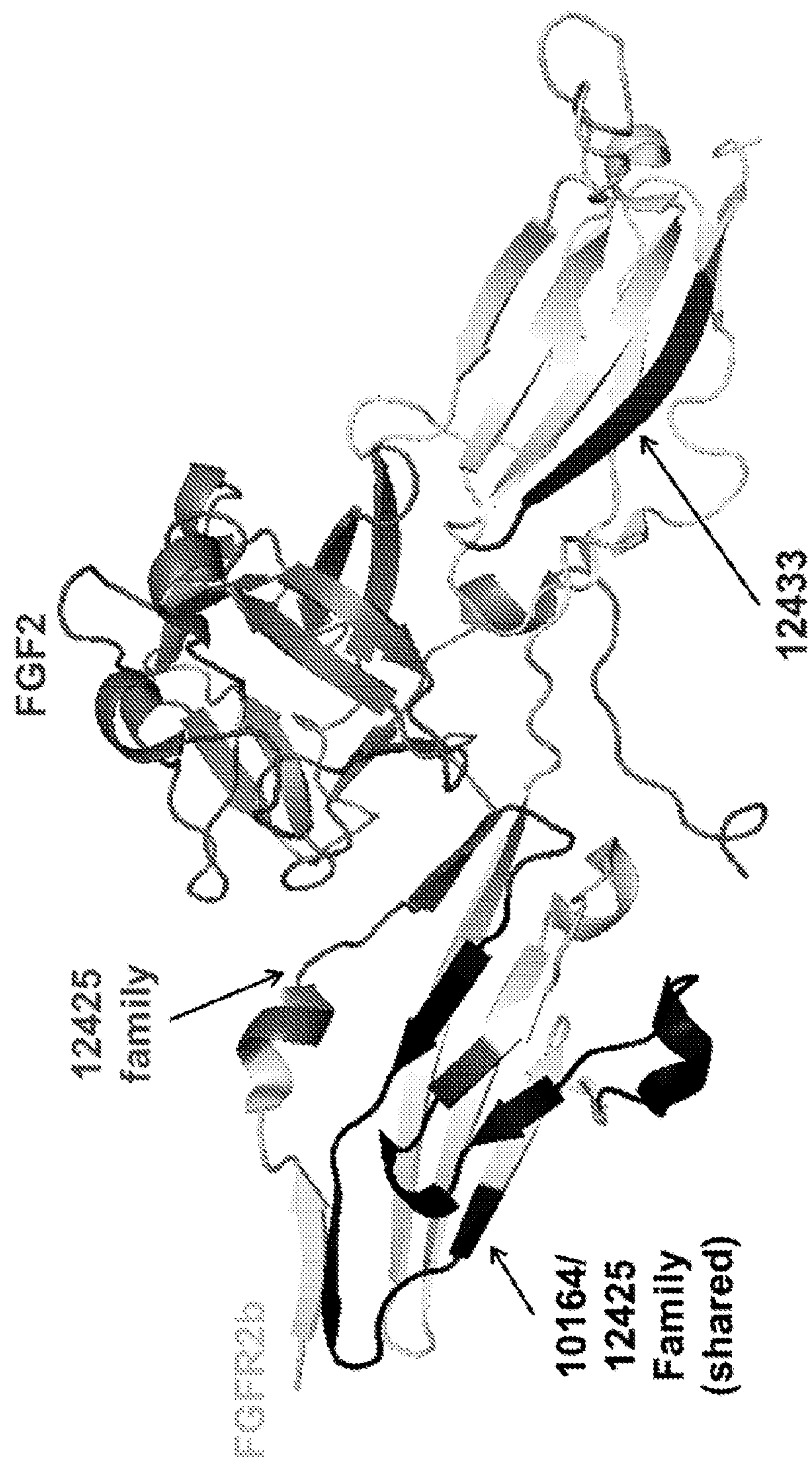
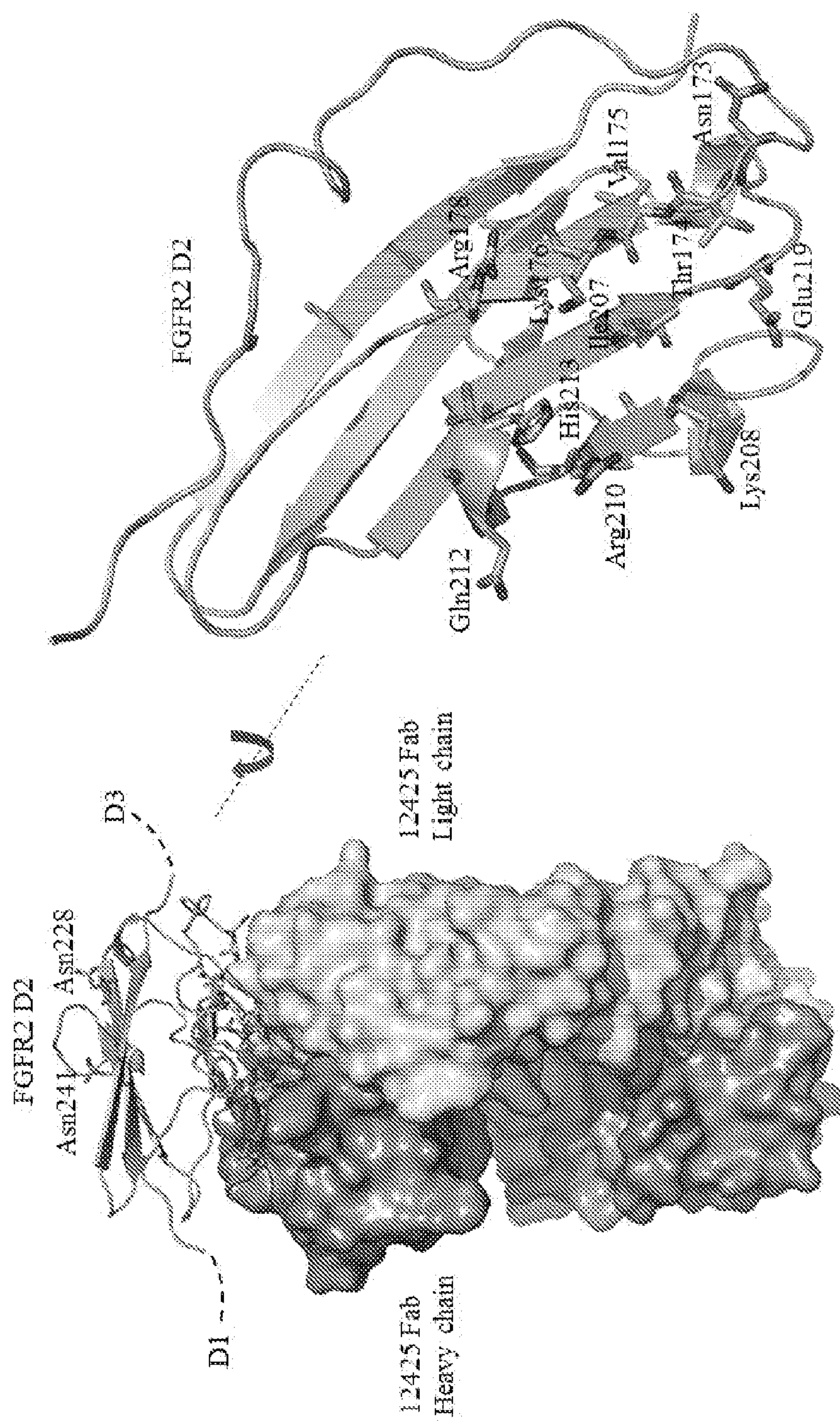


FIG. 20 (A)



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FIG. 20 (B)

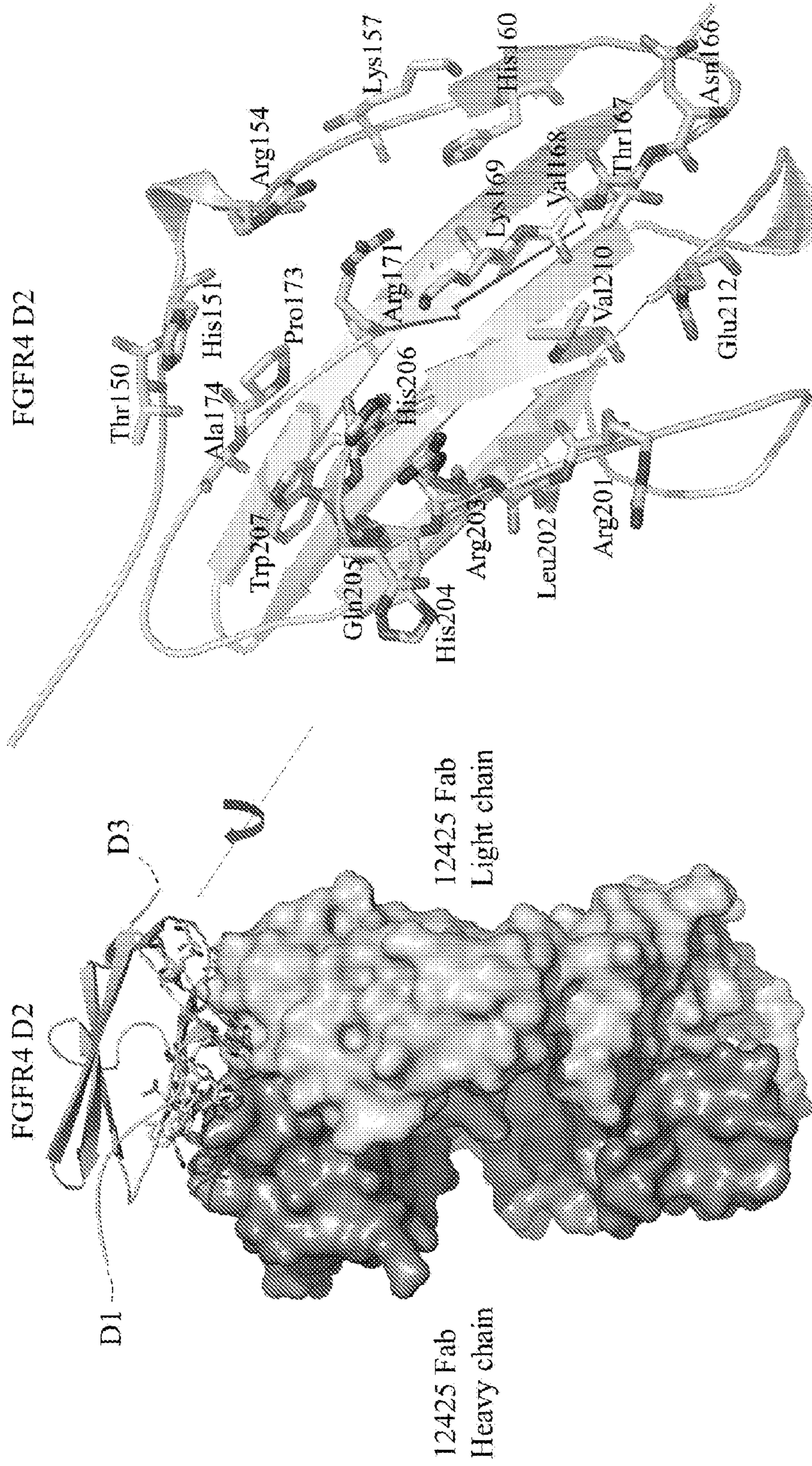


FIG. 20 (C)

	150	160	170	180	190	
Human FGFR2	NSNN	APYWTNT	MEK LHAVPA	ANTVKERCPA	GNEMPTMRWLKNC	
Human FGFR4	HSYP	APYWTHP	MEK LHAVPA	CNTVKERCPA	AGNPTPTIRWLKDC	
	200	210	220	230	240	
Human FGFR2	EKC	HRIGCY	MESEVPSD	CNYTC	YGSINHTYHLDV	
Human FGFR4	PHG	NRIGCI	MESEVPSD	CTYTC	VGSIRYNYLIDV	

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FIG. 21

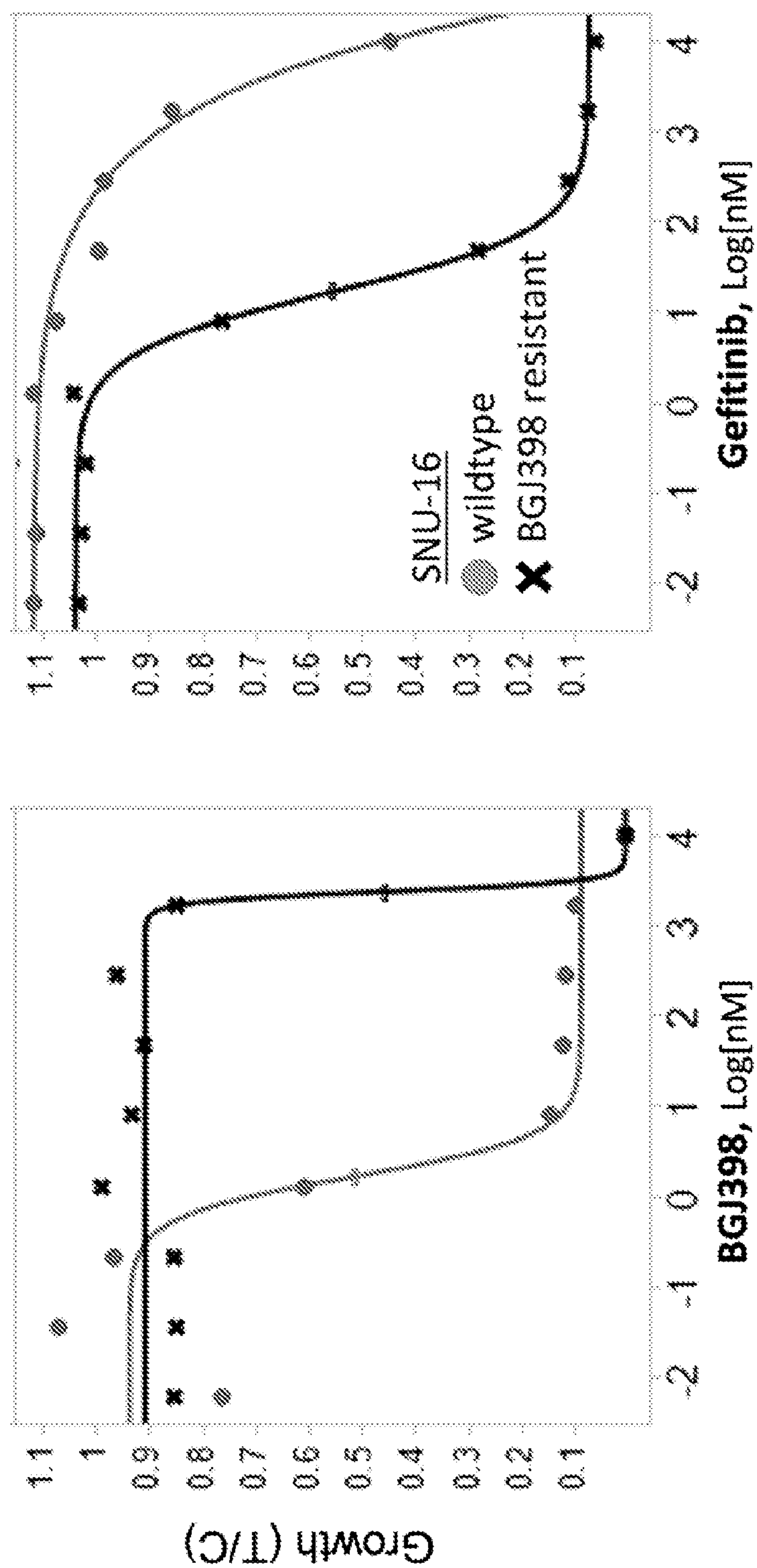


FIG. 1

