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(54) Title: ANTI-FGFR2 ANTIBODIES

(57) Abstract: Monoclonal antibodies that bind and inhibit biological activities of human FGFR2 are disclosed. The antibodies can be used to treat cell proliferative diseases and disorders, including certain forms of cancer, associated with activation or over-expression of FGFR2.

ANTI-FGFR2 ANTIBODIES

CROSS-REFERENCE TO RELATED APPLICATION

[0001] This application claims the benefit of and priority to U.S. Provisional Application Serial No. 61/333,590, filed May 11, 2010; the content of which is incorporated herein in its entirety.

FIELD OF THE INVENTION

[0002] The field of the invention is molecular biology, immunology and oncology. More particularly, the field is antibodies that bind human FGFR2.

BACKGROUND

[0003] Fibroblast Growth Factor Receptor 2 (FGFR2), also known as BEK, BFR-1, CD332, CEK3, CFD1, ECT1, FLJ98662, JWS, KGFR (also known as FGFR2(IIIb)), K-SAM, TK14, and TK25, is one of four highly conserved receptor tyrosine kinases (FGFR1, FGFR2, FGFR3 and FGFR4) that mediate fibroblast growth factor (FGF) signaling by binding FGFs. The FGF receptors are characterized by two or three extracellular immunoglobulin-like domains (IgD1, IgD2 and IgD3), a single-pass transmembrane domain, and a cytoplasmic tyrosine kinase domain. FGF ligand binding induces FGF receptor dimerization and tyrosine autophosphorylation, resulting in cell proliferation, differentiation and migration (Turner *et al.* (2010) NATURE REVIEWS CANCER 10:116-129; Beenken *et al.* (2009) NATURE REVIEWS DRUG DISCOVERY 8:235-254; Gomez-Roman *et al.* (2005) CLIN. CANCER RES. 11:459-65; Chang *et al.* (2005) BLOOD 106:353-6; Eswarakumar *et al.* (2005) CYTOKINE GROWTH FACTOR REV. 16:139-49).

[0004] Alternative splicing in the IgD3 domain yields either the IIIb or IIIc isoform of FGFR1, FGFR2 and FGFR3. The FGFR4 gene is expressed only as the IIIc isoform. The different isoforms of FGF receptors exhibit tissue-specific expression, and they respond to a different spectrum of 18 mammalian FGFs (Beenken *et al.*, *supra*). Binding of FGFs to FGFRs in the presence of heparan sulfate proteoglycans induces autophosphorylation of FGFRs at specific intracellular tyrosine residues. This causes phosphorylation of adaptor molecules, such

as FGFR substrate 2 α (FRS2α), which recruits other proteins to activate various signaling cascades, including the mitogen-activated protein kinase (MAPK) pathway and the phosphoinositide 3-kinase (PI3K)/Akt pathway (Beenken *et al.*, *supra*; Eswarakumar *et al.*, *supra*; Turner *et al.*, *supra*).

[0005] It has been suggested that the dysregulated FGF signaling can directly drive the proliferation of cancer cells, promote the survival of cancer stem cells, and support tumor angiogenesis (Turner *et al.*, *supra*). FGFR2 signaling appears to play a role in cancer. Missense mutations in the FGFR2 gene occur in various cancers, including endometrial cancer (Pollock *et al.*, 2007, ONCOGENE 26:7158-7162; Dutt *et al.*, 2008, PROC. NATL. ACAD. SCI. USA 105:8713-8717), ovarian cancer, breast cancer, lung cancer (Greenman *et al.*, 2007, Nature 446:153-158; Ding *et al.*, 2008, NATURE 455:1069-1075; Davies *et al.*, 2005, CANCER RES. 65:7591-7595) and gastric cancer (Jang *et al.*, 2001, CANCER RES. 61:3541-3543). Some of these activating mutations also have been reported in patients with skeletal disorders (Dutt *et al.*, *supra*). Two independent genome-wide association studies have linked specific single nucleotide polymorphisms (SNPs) in the FGFR2 gene to increased susceptibility to breast cancer (Easton *et al.*, 2007, NATURE 447:1087-1093; Hunter *et al.*, 2007, NAT. GENET. 39:870-874). These cancer-associated SNPs appear to elevate FGFR2 gene expression (Meyer *et al.*, 2008, PLOS BIOL. 6:e108). The FGFR2 gene, located at human chromosome 10q26, is amplified in a subset of breast cancers (Adnane *et al.*, 1991, ONCOGENE 6:659-663; Turner *et al.*, 2010, ONCOGENE 29:2013-2023) and gastric cancer (Hara *et al.*, 1998, LAB. INVEST. 78:1143-1153; Mor *et al.*, 1993, CANCER GENET. CYTOGENET. 65:111-114).

[0006] Naturally occurring antibodies are multimeric proteins that contain four polypeptide chains (FIG. 1). Two of the polypeptide chains are called immunoglobulin heavy chains (H chains), and two of the polypeptide chains are called immunoglobulin light chains (L chains). The immunoglobulin heavy and light chains are connected by an interchain disulfide bond. The immunoglobulin heavy chains are connected by interchain disulfide bonds. A light chain consists of one variable region (V_L in FIG. 1) and one constant region (C_L in FIG. 1). The heavy chain consists of one variable region (V_H in FIG. 1) and at least three constant regions (CH_1, CH_2 and CH_3 in FIG. 1). The variable regions determine the specificity of the antibody. Naturally occurring antibodies have been used as starting material for engineered antibodies, such as chimeric antibodies and humanized antibodies.

[0007] Each variable region contains three hypervariable regions known as complementarity determining regions (CDRs) flanked by four relatively conserved regions known as framework regions (FRs). The three CDRs, referred to as CDR_1, CDR_2, and CDR_3, contribute to the antibody binding specificity.

[0008] Inhibitory antibodies specific against human FGFR2 have been difficult to generate because of the high homology between mouse and human FGFR2. In particular, the ligand binding domain of the mouse and human FGFR2 shares approximately 98% sequence identity (Wei *et al.*, 2006, HYBRIDOMA 25:115-124). Thus, there is a need for improved FGFR2 antibodies that can be used as therapeutic agents.

SUMMARY OF THE INVENTION

[0009] The invention is based on the discovery of a family of antibodies that specifically bind human FGFR2. The antibodies contain FGFR2 binding sites based on the CDRs of an antibody that specifically binds FGFR2. When used as therapeutic agents, the antibodies are engineered, *e.g.*, humanized, to reduce or eliminate an immune response when administered to a human patient.

[0010] The antibodies of the invention prevent or inhibit the activation of (*i.e.*, neutralize) human FGFR2. The antibodies of the invention can be used to inhibit the proliferation of tumor cells *in vitro* or *in vivo*. When administered to a human cancer patient (or an animal model), the antibodies inhibit or reduce tumor growth in the human patient (or animal model).

[0011] These and other aspects and advantages of the invention are illustrated by the following figures, detailed description and claims. As used herein, "including" means without limitation, and examples cited are non-limiting.

DESCRIPTION OF THE DRAWINGS

[0012] The invention can be more completely understood with reference to the following drawings.

[0013] **FIG. 1** (prior art) is a schematic representation of a typical antibody.

[0014] **FIG. 2** is a graph summarizing results from an experiment to measure stimulation of proliferation of FGFR2-IIIb-expressing FDCP-1 cells by FGF2 (●), FGF7 (▽), FGF9 (□) and FGF10 (x).

[0015] **FIG. 3** is a graph summarizing results from an experiment to measure stimulation of proliferation of FGFR2-IIIc-expressing FDCP-1 cells by FGF2 (●), FGF7 (▽), FGF9 (□) and FGF10 (x).

[0016] **FIG. 4** is a graph summarizing results from an experiment to measure inhibition of proliferation of FDCP-1 cells expressing wild type FGFR2-IIIb (□), wild type FGFR2-IIIc(▽), or truncated FGFR2-IIIb (*), by treatment with antibody 4B9.

[0017] **FIG. 5** is a graph summarizing results from an experiment to measure inhibition of proliferation of FDCP-1 cells expressing wild type FGFR2-IIIb (□), FGFR2-IIIb S252W (■), or FGFR2-IIIb N550K (▲), by treatment with antibody 4B9.

[0018] **FIG. 6** is a graph summarizing results from an experiment to measure inhibition of growth of SNU-16 xenograft tumors by treatment with antibody 4B9 at 2 mg/kg (also referred to herein as "mpk") (○), 5 mpk (Δ), 10 mpk (x) or 20 mpk (*), with mIgG at 20 mpk (♦) serving as a negative control.

[0019] **FIG. 7** is a graph summarizing results from an experiment to measure the effect of antibody 4B9 (○) on the *in vivo* growth of FGFR2-amplified breast cancer cell line MFM-223 (murine IgG (♦)).

[0020] **FIG. 8** is a schematic diagram showing the amino acid sequences of the complete murine immunoglobulin heavy chain variable region of 4B9 (SEQ ID NO: 2) and the complete humanized heavy chain variable regions denoted as Hu4B9-65 (SEQ ID NO: 35) and Hu4B9-82, -83 (SEQ ID NO: 37). The amino acid sequences for each heavy chain variable region are aligned against one another, and Complementary Determining Sequences (CDR) (Kabat definition), CDR_1, CDR_2, and CDR_3, are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0021] **FIG. 9** is a schematic diagram showing the CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) for each of the variable region sequences shown in **FIG. 8**.

[0022] **FIG. 10** is a schematic diagram showing the amino acid sequences of the complete murine immunoglobulin light chain variable region of 4B9 (SEQ ID NO: 4) and the complete humanized light chain variable regions denoted as Hu4B9-65 (SEQ ID NO: 40), Hu4B9-82 (SEQ ID NO: 44), and Hu4B9-83 (SEQ ID NO: 46). The amino acid sequences for each light chain variable region are aligned against one another, and CDR_1, CDR_2, and CDR_3 sequences

(Kabat definition) are identified in boxes. The unboxed sequences represent framework (FR) sequences.

[0023] **FIG. 11** is a schematic diagram showing the CDR_1, CDR_2, and CDR_3 sequences (Kabat definition) for each of the variable region sequences shown in **FIG. 10**.

[0024] **FIG. 12** is a graph summarizing results from an experiment to measure inhibition of proliferation of FDCP-1 cells expressing wild type FGFR2-IIIb by treatment with antibody 4B9 (□), Hu4B9-65 (▲), Hu4B9-82 (▼) and Hu4B9-83 (♦).

DETAILED DESCRIPTION

[0025] The FGFR2 antibodies of the invention are based on the antigen binding sites of a monoclonal antibody selected on the basis of neutralizing the biological activity of human FGFR2 polypeptides. The antibodies contain immunoglobulin variable region CDR sequences that define a binding site for human FGFR2.

[0026] Because of the neutralizing activity of these antibodies, they are useful for inhibiting the growth and/or proliferation of certain cancer cells and tumors. The antibodies can be engineered to minimize or eliminate an immune response when administered to a human patient. Various features and aspects of the invention are discussed in more detail below.

[0027] As used herein, unless otherwise indicated, the term "antibody" means an intact antibody (*e.g.*, an intact monoclonal antibody) or antigen-binding fragment of an antibody (*e.g.*, an antigen-binding fragment of a monoclonal antibody), including an intact antibody or antigen-binding fragment that has been modified, engineered or chemically conjugated. Examples of antibodies that have been modified or engineered are chimeric antibodies, humanized antibodies, and multispecific antibodies (*e.g.*, bispecific antibodies). Examples of antigen-binding fragments include Fab, Fab', $F(ab')_2$, Fv, single chain antibodies (*e.g.*, scFv) and diabodies. An antibody conjugated to a toxin moiety is an example of a chemically conjugated antibody.

Antibodies that Bind Human FGFR2

[0028] Antibodies of the invention comprise: (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3} and (b) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, wherein the heavy chain

variable region and the light chain variable region together define a single binding site for binding human FGFR2.

[0029] As disclosed herein, an antibody may comprise: (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3} and (b) immunoglobulin light chain variable region, wherein the heavy chain variable region and the light chain variable region together define a single binding site for binding human FGFR2. A CDR_{H1} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (**4B9; Hu4B9-65; Hu4B9-82, -83**), SEQ ID NO: 7 (**4B9; Hu4B9-65**), and SEQ ID NO: 47 (**Hu4B9-82, -83**); a CDR_{H2} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 6 (**4B9; Hu4B9-65**) and SEQ ID NO: 38 (**Hu4B9-82, -83**); and a CDR_{H3} comprises an amino acid sequence selected from the group consisting of amino acid sequence FDY (**4B9; Hu4B9-65; Hu4B9-82, -83**) and SEQ ID NO: 11 (**4B9; Hu4B9-65; Hu4B9-82, -83**). Throughout the specification a particular SEQ ID NO. is followed in parentheses by the antibody that was the origin of that sequence. For example, "SEQ ID NO: 47 (**Hu4B9-82, -83**)" means that SEQ ID NO: 47 comes from the humanized antibody 4B9 denoted Hu4B9-82, -83.

[0030] In some embodiments, the heavy chain variable region comprises a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 5 or SEQ ID NO: 7 (**4B9; Hu4B9-65; Hu4B9-82, -83**), a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6 (**4B9; Hu4B9-65**), and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 11 (**4B9; Hu4B9-65; Hu4B9-82, -83**).

[0031] In some embodiments, the heavy chain variable region comprises a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 5 (**4B9; Hu4B9-65; Hu4B9-82, -83**) or SEQ ID NO: 47 (**Hu4B9-82, -83**), a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 38 (**Hu4B9-82, -83**), and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 11 (**4B9; Hu4B9-65; Hu4B9-82, -83**).

[0032] Preferably, the CDR_{H1}, CDR_{H2}, and CDR_{H3} sequences are interposed between human or humanized immunoglobulin FRs. The antibody can be an intact antibody or an antigen-binding antibody fragment.

[0033] In other embodiments, the antibody comprises (a) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, and (b) an immunoglobulin heavy chain variable region, wherein the IgG light chain variable region and the IgG heavy

chain variable region together define a single binding site for binding human FGFR2. A CDR_{L1} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 12 (**4B9**) and SEQ ID NO: 41 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**); a CDR_{L2} comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 13 (**4B9**) and SEQ ID NO: 42 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**); and a CDR_{L3} comprises an amino acid sequence of SEQ ID NO: 14 (**4B9; Hu4B9-65; Hu4B9-82; Hu4B9-83**).

[0034] In some embodiments, the light chain variable region comprises a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 12 (**4B9**); a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 13 (**4B9**); and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 14 (**4B9; Hu4B9-65; Hu4B9-82; Hu4B9-83**).

[0035] In some embodiments, the light chain variable region comprises a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 41 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**); a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 42 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**); and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 14 (**4B9; Hu4B9-65; Hu4B9-82; Hu4B9-83**).

[0036] Preferably, the CDR_{L1}, CDR_{L2}, and CDR_{L3} sequences are interposed between human or humanized immunoglobulin FRs. The antibody can be an intact antibody or an antigen-binding antibody fragment.

[0037] In some embodiments, the antibody comprises: (a) an immunoglobulin heavy chain variable region comprising the structure CDR_{H1}-CDR_{H2}-CDR_{H3} and (b) an immunoglobulin light chain variable region comprising the structure CDR_{L1}-CDR_{L2}-CDR_{L3}, wherein the heavy chain variable region and the light chain variable region together define a single binding site for binding human FGFR2. The CDR_{H1} is an amino acid sequence selected from the group consisting of SEQ ID NO: 5 or SEQ ID NO: 7 (**4B9; Hu4B9-65; Hu4B9-82, -83**); the CDR_{H2} is an amino acid sequence selected from the group consisting of SEQ ID NO: 6 (**4B9; Hu4B9-65**) and SEQ ID NO: 38 (**Hu4B9-82, -83**); and the CDR_{H3} is an amino acid sequence selected from the group consisting of amino acid sequence FDY and SEQ ID NO: 11 (**4B9; Hu4B9-65; Hu4B9-82, -83**). The CDR_{L1} is an amino acid sequence selected from the group consisting of SEQ ID NO: 12 (**4B9**) and SEQ ID NO: 41 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**); the CDR_{L2} is an amino acid sequence selected from the group consisting of SEQ ID NO: 13 (**4B9**) and SEQ

ID NO: 42 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**); and the CDR_{L3} comprises the amino acid sequence of SEQ ID NO: 14 (**4B9; Hu4B9-65; Hu4B9-82; Hu4B9-83**).

[0038] In another embodiment, the antibody comprises an immunoglobulin heavy chain variable region selected from the group consisting of SEQ ID NO: 2 (**4B9**), SEQ ID NO: 35 (**Hu4B9-65**), and SEQ ID NO: 37 (**Hu4B9-82, -83**), and an immunoglobulin light chain variable region selected from the group consisting of SEQ ID NO: 4 (**4B9**), SEQ ID NO: 40 (**Hu4B9-65**), SEQ ID NO: 44 (**Hu4B9-82**) and SEQ ID NO: 46 (**Hu4B9-83**).

[0039] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2 (**4B9**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 4 (**4B9**).

[0040] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 (**Hu4B9-65**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 40 (**Hu4B9-65**).

[0041] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 (**Hu4B9-82, -83**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 44 (**Hu4B9-82**).

[0042] In some embodiments, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 (**Hu4B9-82, -83**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 46 (**Hu4B9-83**).

[0043] In other embodiments, the antibody comprises (i) an immunoglobulin heavy chain selected from the group consisting of SEQ ID NO: 21 (**4B9**), SEQ ID NO: 54 (**Hu4B9-65**), and SEQ ID NO: 56 (**Hu4B9-82, -83**), and (ii) an immunoglobulin light chain selected from the group consisting of SEQ ID NO: 23 (**4B9**), SEQ ID NO: 58 (**Hu4B9-65**), SEQ ID NO: 60 (**Hu4B9-82**) and SEQ ID NO: 62 (**Hu4B9-83**).

[0044] In certain embodiments, the antibody comprises (i) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 21 **(4B9)**, and (ii) an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 23 **(4B9)**.

[0045] In certain embodiments, the antibody comprises (i) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 54 **(Hu4B9-65)**, and (ii) an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 58 **(Hu4B9-65)**.

[0046] In certain embodiments, the antibody comprises (i) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 56 **(Hu4B9-82, -83)**, and (ii) an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 60 **(Hu4B9-82)**.

[0047] In certain embodiments, the antibody comprises (i) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 56 **(Hu4B9-82, -83)**, and (ii) an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 62 **(Hu4B9-83)**.

[0048] In other embodiments, an isolated antibody that binds human FGFR2 comprises an immunoglobulin heavy chain variable region comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the entire variable region or the framework region sequence of SEQ ID NO: 2 **(4B9)**, SEQ ID NO: 35 **(Hu4B9-65)**, and SEQ ID NO: 37 **(Hu4B9-82, -83)**.

[0049] In other embodiments, an isolated antibody that binds human FGFR2 comprises an immunoglobulin light chain variable region comprising an amino acid sequence that is at least 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the entire variable region or the framework region sequence of SEQ ID NO: 4 **(4B9)**, SEQ ID NO: 40 **(Hu4B9-65)**, SEQ ID NO: 44 **(Hu4B9-82)** and SEQ ID NO: 46 **(Hu4B9-83)**.

[0050] Homology or identity may be determined in various ways that are within the skill in the art, for instance, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, (1990) PROC. NATL. ACAD. SCI. USA 87, 2264-2268; Altschul, (1993) J. MOL.

EVOL. 36, 290-300; Altschul *et al.*, (1997) NUCLEIC ACIDS RES. 25, 3389-3402, incorporated by reference) are tailored for sequence similarity searching. The approach used by the BLAST program is to first consider similar segments between a query sequence and a database sequence, then to evaluate the statistical significance of all matches that are identified and finally to summarize only those matches which satisfy a preselected threshold of significance. For a discussion of basic issues in similarity searching of sequence databases see Altschul *et al.*, (1994) NATURE GENETICS 6, 119-129 which is fully incorporated by reference. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (*i.e.*, the statistical significance threshold for reporting matches against database sequences), cutoff, matrix and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, (1992) PROC. NATL. ACAD. SCI. USA 89, 10915-10919, fully incorporated by reference). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent Blastp parameter settings may be Q=9; R=2; wink=1; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (e.g.: -G, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; -E, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default = -3; -r, reward for nucleotide match [Integer]: default = 1; -e, expect value [Real]: default = 10; -W, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; -y, Dropoff (X) for blast extensions in bits: default = 20 for blastn/ 7 for others; -X, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to blastn; and –Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may also be used (default parameters may include, *e.g.*, Blosum62 matrix and Gap Opening Penalty = 10 and Gape Extenstion Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty) and the equivalent settings in protein comparisons are GAP=8 and LEN=2.

[0051] In each of the foregoing embodiments, it is contemplated herein that immunoglobulin heavy chain variable region sequences and/or light chain variable region sequences that together bind human FGFR2 may contain amino acid alterations (*e.g.*, at least 1, 2, 3, 4, 5, or 10 amino acid substitutions, deletions, or additions) in the framework regions of the heavy and/or light chain variable regions.

[0052] In some embodiments, an isolated antibody binds human FGFR2 with a K_D of 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 950 pM, 900 pM, 850 pM, 800 pM, 750 pM, 700 pM, 650 pM, 600 pM, 550 pM, 500 pM, 450 pM, 400 pM, 350 pM, 300 pM, 250 pM, 200 pM, 150 pM, 100 pM, 50 pM or lower. Unless otherwise specified, K_D values are determined by surface plasmon resonance methods under the conditions described, for example, in Examples 5 and 9.

Production of Antibodies

[0053] Methods for producing antibodies of the invention are known in the art. For example, DNA molecules encoding light chain variable regions and heavy chain variable regions can be chemically synthesized using the sequence information provided herein. Synthetic DNA molecules can be ligated to other appropriate nucleotide sequences, including, *e.g.*, constant region coding sequences, and expression control sequences, to produce conventional gene expression constructs encoding the desired antibody. Production of defined gene constructs is within routine skill in the art. Alternatively, the sequences provided herein can be cloned out of hybridomas by conventional hybridization techniques or polymerase chain reaction (PCR) techniques, using synthetic nucleic acid probes whose sequences are based on sequence information provided herein, or prior art sequence information regarding genes encoding the heavy and light chains of murine antibodies in hybridoma cells.

[0054] Nucleic acids encoding desired antibodies can be incorporated (ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and myeloma cells that do not otherwise produce IgG protein. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light or heavy chain variable regions.

[0055] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, *e.g.*, Trp or Tac, and a prokaryotic signal sequence. The expressed secreted protein accumulates in refractile or inclusion bodies, and can be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the proteins refolded and cleaved by methods known in the art.

[0056] If the engineered gene is to be expressed in eukaryotic host cells, *e.g.*, CHO cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, IgG enhancers, and various introns. This expression vector optionally contains sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy or light chain to be expressed. The gene construct can be introduced into eukaryotic host cells using convention techniques. The host cells express V_L or V_H fragments, V_L-V_H heterodimers, V_H-V_L or V_L-V_H single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof, each of which may be attached to a moiety having another function (*e.g.*, cytotoxicity). In some embodiments, a host cell is transfected with a single vector expressing a polypeptide expressing an entire, or part of, a heavy chain (*e.g.*, a heavy chain variable region) or a light chain (*e.g.*, a light chain variable region). In other embodiments, a host cell is transfected with a single vector encoding (a) a polypeptide comprising a heavy chain variable region and a polypeptide comprising a light chain variable region, or (b) an entire immunoglobulin heavy chain and an entire immunoglobulin light chain. In still other embodiments, a host cell is co-transfected with more than one expression vector (*e.g.*, one expression vector expressing a polypeptide comprising an entire, or part of, a heavy chain or heavy chain variable region, and another expression vector expressing a polypeptide comprising an entire, or part of, a light chain or light chain variable region).

[0057] A polypeptide comprising an immunoglobulin heavy chain variable region or a light chain variable region can be produced by growing a host cell transfected with an expression vector encoding such variable region, under conditions that permit expression of the polypeptide. Following expression, the polypeptide can be harvested and purified using techniques well known in the art, *e.g.*, affinity tags such as glutathione-S-transferase (GST) and histidine tags.

[0058] A monoclonal antibody that binds human FGFR2, or an antigen-binding fragment of the antibody, can be produced by growing a host cell transfected with: (a) an expression vector that encodes a complete or partial immunoglobulin heavy chain, and a separate expression vector that encodes a complete or partial light chain; or (b) a single expression vector that encodes both chains (*e.g.*, complete or partial heavy and light chains) under conditions that permit expression of both chains. The intact antibody (or the antigen-binding fragment of the antibody) can be harvested and purified using techniques well known in the art, *e.g.*, Protein A, Protein G, affinity tags such as glutathione-S-transferase (GST) and histidine tags. It is within ordinary skill in the art to express the heavy chain and the light chain from a single expression vector or from two separate expression vectors.

Modifications to the Antibodies

[0059] Methods for reducing or eliminating the antigenicity of antibodies and antibody fragments are known in the art. When the antibodies are to be administered to a human, the antibodies preferably are "humanized" to reduce or eliminate antigenicity in humans. Preferably, the humanized antibodies have the same, or substantially the same, affinity for the antigen as the non-humanized mouse antibody from which it was derived.

[0060] In one humanization approach, chimeric proteins are created in which mouse immunoglobulin constant regions are replaced with human immunoglobulin constant regions. See, *e.g.*, Morrison *et al.*,1984, PROC. NAT. ACAD. SCI. 81:6851-6855, Neuberger *et al.*, 1984, NATURE 312:604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,816,567 (Cabilly).

[0061] In an approach known as CDR grafting, the CDRs of the light and heavy chain variable regions are grafted into frameworks from another species. For example, murine CDRs can be grafted into human FRs. In some embodiments of the invention, the CDRs of the light and heavy chain variable regions of an anti-FGFR2 antibody are grafted into human FRs or consensus human FRs. To create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. CDR grafting is described in U.S. Patent Nos. 7,022,500 (Queen); 6,982,321 (Winter); 6,180,370 (Queen); 6,054,297 (Carter); 5,693,762 (Queen); 5,859,205 (Adair); 5,693,761 (Queen); 5,565,332 (Hoogenboom); 5,585,089 (Queen); 5,530,101 (Queen); Jones *et al.* (1986)

NATURE 321: 522-525; Riechmann *et al.* (1988) NATURE 332: 323-327; Verhoeyen *et al.* (1988) SCIENCE 239: 1534-1536; and Winter (1998) FEBS LETT 430: 92-94.

[0062] In an approach called "SUPERHUMANIZATION™," human CDR sequences are chosen from human germline genes, based on the structural similarity of the human CDRs to those of the mouse antibody to be humanized. See, *e.g.*, U.S. Patent No. 6,881,557 (Foote); and Tan *et al.*, 2002, J. IMMUNOL 169:1119-1125.

[0063] Other methods to reduce immunogenicity include "reshaping," "hyperchimerization," and "veneering/resurfacing." See, *e.g.*, Vaswami *et al.*,1998, ANNALS OF ALLERGY, ASTHMA, & IMMUNOL. 81:105; Roguska *et al.*, 1996, PROT. ENGINEER 9:895-904; and U.S. Patent No. 6,072,035 (Hardman). In the veneering/resurfacing approach, the surface accessible amino acid residues in the murine antibody are replaced by amino acid residues more frequently found at the same positions in a human antibody. This type of antibody resurfacing is described, *e.g.*, in U.S. Patent No. 5,639,641 (Pedersen).

[0064] Another approach for converting a mouse antibody into a form suitable for medical use in humans is known as ACTIVMAB™ technology (Vaccinex, Inc., Rochester, NY), which involves a vaccinia virus-based vector to express antibodies in mammalian cells. High levels of combinatorial diversity of IgG heavy and light chains are said to be produced. See, *e.g.*, U.S. Patent Nos. 6,706,477 (Zauderer); 6,800,442 (Zauderer); and 6,872,518 (Zauderer).

[0065] Another approach for converting a mouse antibody into a form suitable for use in humans is technology practiced commercially by KaloBios Pharmaceuticals, Inc. (Palo Alto, CA). This technology involves the use of a proprietary human "acceptor" library to produce an "epitope focused" library for antibody selection.

[0066] Another approach for modifying a mouse antibody into a form suitable for medical use in humans is HUMAN ENGINEERING™ technology, which is practiced commercially by XOMA (US) LLC. See, *e.g.*, PCT Publication No. WO 93/11794 and U.S. Patent Nos. 5,766,886; 5,770,196; 5,821,123; and 5,869,619.

[0067] Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of an antibody disclosed herein.

[0068] If the antibody is for use as a therapeutic agent, it can be conjugated to an effector moiety such as a small molecule toxin or a radionuclide using standard *in vitro* conjugation

chemistries. If the effector moiety is a polypeptide, the antibody can be chemically conjugated to the effector or joined to the effector as a fusion protein. Construction of fusion proteins is within ordinary skill in the art.

Use of Antibodies

[0069] Antibodies disclosed herein can be used to treat various forms of cancer, *e.g.*, breast, ovarian, prostate, cervical, colorectal, lung, pancreatic, gastric, and head and neck cancers. The cancer cells are exposed to a therapeutically effective amount of the antibody so as to inhibit or reduce proliferation of the cancer cells. In some embodiments, the antibodies inhibit cancer cell proliferation by at least 40%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, 99%, or 100%.

[0070] In some embodiments, the disclosed antibodies can be used in a method to inhibit tumor growth in a human patient. The method comprises administering to the patient a therapeutically effective amount of the antibody. Cancers associated with FGFR2 overexpression and/or activation include breast cancer, ovarian cancer, prostate cancer, cervical cancer, lung cancer, some forms of brain cancer, melanomas, and gastrointestinal cancers (*e.g.*, colorectal, pancreatic, gastric, head and neck).

[0071] As used herein, "treating" a disease means: (a) reducing symptoms of the disease; (b) inhibiting progression of the disease; (c) causing regression of the disease; or (d) curing the disease.

[0072] Generally, a therapeutically effective amount of active component is in the range of 0.1 mg/kg to 100 mg/kg, *e.g.*, 1 mg/kg to 100 mg/kg, 1 mg/kg to 10 mg/kg. The amount administered will depend on variables such as the type and extent of disease or indication to be treated, the overall health of the patient, the *in vivo* potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue level. Alternatively, the initial dosage can be smaller than the optimum, and the daily dosage may be progressively increased during the course of treatment. Human dosage can be optimized, *e.g.*, in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount and the disease being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. A preferred route of administration is parenteral, *e.g.*, intravenous infusion. Formulation of monoclonal antibody-based drugs is within ordinary skill

in the art. In some embodiments of the invention a monoclonal antibody is lyophilized and reconstituted in buffered saline at the time of administration.

[0073] For therapeutic use, an antibody preferably is combined with a pharmaceutically acceptable carrier. As used herein, "pharmaceutically acceptable carrier" means buffers, carriers, and excipients suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio. The carrier(s) should be "acceptable" in the sense of being compatible with the other ingredients of the formulations and not deleterious to the recipient. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[0074] Pharmaceutical compositions containing antibodies of the invention can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intradermal, inhalation, transdermal, topical, transmucosal, and rectal administration. A preferred route of administration for monoclonal antibodies is IV infusion. Useful formulations can be prepared by methods well known in the pharmaceutical art. For example, see *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990). Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

[0075] For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyetheylene glycol), and suitable mixtures thereof.

[0076] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished, for example, by filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

EXAMPLES

[0077] The following Examples are merely illustrative and are not intended to limit the scope or content of the invention in any way.

Example 1: Cell Lines and Reagents

[0078] KATO III, HEC-1-A, AN3 CA, SNU-16, and human lung cancer cell lines were acquired from the American Type Culture Collection (Rockville, MD). FDCP-1 and Ba/F3, MFM-223, MFE-296, MFE-280, MFE-319 and ESS-1 cells were obtained from the German Collection of Microorganisms and Cell Cultures. All human cell lines were cultured according to the instructions specified by the suppliers, at 37°C, in an atmosphere containing 5% CO_2. All FGFs were purchased from R&D Systems, Inc. (Minneapolis, MN).

[0079] To establish cell-based assays to screen for functional FGFR2 antibodies, we first engineered Ba/F3 and FDCP-1 cells to express wild type FGFR2 and cancer-associated mutants or variants of FGFR2. FGFR-driven FDCP cells and Ba/F3 cells were obtained by the following methods. FDCP-1 cells were transfected by electroporation with plasmids encoding the IIIb, IIIc isoform or C-terminally truncated variant of human FGFR2 as well as cancer-associated FGFR2-IIIb S252W, or FGFR2-IIIb N550K mutants. Following selection with G418 (600 μg/ml), single clones were isolated and tested for their FGF1-dependent proliferation in the absence of IL3 by the MTT [3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide] assay (Sigma-Aldrich, St. Louis, MO). MTT reagent (10 μl) was added to the cells and the reaction was stopped with 100 μl of 10% SDS with 2N HCL after four hours. The plates were analyzed the following day. The clones that exhibited robust FGF-1-dependent proliferation in the absence of IL3 were used for subsequent studies. To generate retroviruses expressing FGFR2, cDNAs encoding various human FGFR2 variants were each inserted into a retroviral vector. Retroviruses were produced by transfecting Phoenix cells using Lipofectamine 2000 (Invitrogen, Carlsbad, CA). Supernatants containing the retroviruses were used to infect Ba/F3 cells by centrifugation at 2500 rpm for 90 minutes, in the presence of

8 μg/ml of polybrene (Sigma-Aldrich). Individual clones were isolated by limiting dilution, and cell surface receptor expression was verified by flow cytometry.

[0080] Cancer cell lines with FGFR amplification were identified as follows. The CGP copy number database at the Wellcome Trust Sanger Institute (www.sanger.ac.uk) was queried for *FGFR2* amplification (gene copy number > 7). The copy number of the cell lines with potential *FGFR2* amplification was analyzed by quantitative PCR (qPCR) using FGFR2 specific primers (5'-ACTTGGGCTGGAGTGATTTG-3' (SEQ ID NO: 24) and 5'-AATCCCATCTGCACACTTCC-3' (SEQ ID NO: 25)) and reference gene (transketolase) primers (5'-CAAAAACATGGCTGAGCAGA-3' (SEQ ID NO: 26) and 5'-GAAACAGGCCCCACTTTGTA-3' (SEQ ID NO: 27)). The FGFR2 gene copy number was calculated essentially as described in Toyokawa *et al.*, 2009, ONCOL. REP. 21:875-880 .

[0081] FGFR gene expression analysis was performed as follows. Total RNA was isolated by the RNeasy™ mini kit (Qiagen, Valencia, CA). Quantitative RT-PCR (qRT-PCR) was performed using a QuantiTect™ SYBR Green RT-PCR kit (Qiagen), with primers specific for FGFR2, FGFR2-IIIb, FGFR2-IIIc, and HPRT. The expression levels were normalized to HPRT.

[0082] Previous studies have demonstrated that ectopic expression of FGFRs in murine pro-B Ba/F3 or bone marrow FDCP-1 cells confers FGF1-dependent proliferation in the absence of IL-3 (Tannheimer *et al.*, 2000, BREAST CANCER RES. 2:311-320; Ornitz *et al.*, 1996, J. BIOL. CHEM. 271:15292-15297). As expected, there was no noticeable proliferation of FDCP-1 cells stably expressing wild-type FGFR2 in the absence of IL-3 and FGF1. It was known that FGF1, 3, 7, 10 and 22 transduce signals through FGFR2-IIIb, and that FGFR2-IIIc responds to a broader panel of ligands including FGF1, 2, 4, 6, 9, 16, 17, 18 and 20 (Tannheimer et al., supra; Ornitz *et al.*, supra; Zhang *et al.*, 2006, J. BIOL. CHEM. 281:15964-15700). The proliferation of FDCP-1 cells expressing the IIIb isoform of FGFR2 was stimulated by FGF7 and FGF10, but not by FGF2 and FGF9 (**FIG. 2**). The proliferation of cells expressing the IIIc isoform was enhanced by FGF2 and FGF9 specifically (**FIG. 3**).

Example 2: Production of Anti-FGFR2 Monoclonal Antibodies

[0083] Mice were immunized with a 1:1 mixture of human FGFR2 IgD2-IgD3 (IIIb) and human FGFR2 IgD2-IgD3 (IIIc) fused with a human Fc moiety at their C-termini. Mouse

immunizations and cell fusions were performed by a commercial vendor (Precision Antibody, Columbia, MD).

[0084] In a primary screen, hybridoma supernatants were screened to detect binding to human FGFR2 IgD2-IgD3, using an ELISA format. Antibodies that passed the primary screen were subjected to a secondary screen, which was a cell-based proliferation assay described in Example 3 (below).

[0085] The primary screen was performed using the supernatants of the murine hybridoma clones yielded from the splenic fusion of the mice immunized with the extracellular domain of human FGFR2. Assay plates were coated with 100 ng/well of recombinant soluble FGFR2 extracellular domain and then blocked with 5% milk in PBS for one hour at room temperature. Then 50 µl of hybridoma supernatant was added to each well to allow antibody binding for one hour at room temperature. Plates were washed three times with wash buffer (PBS with 0.1% Tween 20) followed by incubation with a HRP-conjugated goat anti-mouse IgG heavy and light chain secondary antibody. The assay was developed using TMB (tetramethylbenzene) as a substrate, and absorbance was read at 620 nm.

Example 3: Identification of FGFR2 Antagonist Antibodies

[0086] To screen for FGFR2 antagonist antibodies, hybridoma supernatants containing FGFR2 antibodies were added to FDCP cells ectopically expressing one of the following five forms of FGFR2: (1) wild type FGFR2-IIIb; (2) wild type FGFR2-IIIc; (3) FGFR2-III(b) S252W; (4) FGFR2-III(b) N550K; and (5) FGFR2-III(b) with C-terminal truncation. The supernatants were added to the FGFR2-expressing cells at a 1:1 ratio (volume) in a flat-bottomed 96-well plate (70,000 cells/ well) with heparin (5 µg/ml) ± FGF1 (8 ng/ml). After incubation at 37°C for 2 days, MTT assays were conducted as described above.

[0087] The supernatant of clone 4B9 demonstrated potent and selective inhibition of the FDCP-1 proliferation driven by the IIIb-isoform of FGFR2. Antibody 4B9 (also referred to as antibody GP369), produced by clone 4B9, was purified by conventional techniques for further characterization. Surface plasmon resonance analysis indicated that antibody 4B9 exhibited strong affinity towards human FGFR2-IIIb and showed no detectable binding to the human FGFR2-IIIc. No binding of antibody 4B9 to human FGFR1-IIIc or FGFR3-IIIb was detected.

Example 4: Sequence Analysis

[0088] The light chain isotype and heavy chain isotype of antibody 4B9 in Example 1 was determined using the IsoStrip™ Mouse Monoclonal Antibody Isotyping Kit according to the manufacturer's instructions (Roche Applied Science, Indianapolis, IN). The antibody was determined to be Kappa light chain and IgG1 heavy chain.

[0089] The heavy and light chain variable regions of antibody 4B9 were sequenced using 5' RACE (Rapid Amplification of cDNA Ends). Total RNA was extracted from the 4B9 monoclonal hybridoma cell line using the RNeasy™ Miniprep kit according to the vendor's instructions (Qiagen, Valencia, CA). Full-length first strand cDNA containing 5' ends was generated using SMARTer™ RACE cDNA Amplification Kit (Clontech, Palo Alto, CA) according to the manufacturer's instructions using random primers for 5' RACE.

[0090] The variable regions of the kappa and heavy IgG1 chains were amplified by PCR, using KOD Hot Start™ Polymerase (EMD Chemicals, Gibbstown, NJ) according to the manufacturer's instructions. For amplification of 5' cDNA ends in conjunction with the SMARTer™ RACE cDNA Amplification Kit , the Universal Primer Mix A primer (Clontech), a mix of 5'CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT 3' (SEQ ID NO: 28) and 5' CTAATACGACTCACTATAGGGC 3' (SEQ ID NO: 29), was used as a 5' primer. The heavy chain variable region was amplified using the above 5' primers and a 3' IgG1 constant region specific primer, 5' TATGCAAGGCTTACAACCACA 3' (SEQ ID NO: 30). The kappa chain variable region was amplified with the above 5' primers and a 3' kappa constant region specific primer, CGACTGAGGCACCTCCAGATGTT 3' (SEQ ID NO: 31).

[0091] Individual PCR products were isolated by agarose gel electrophoresis and purified using the Qiaquick™ Gel Purification kit according to the manufacturer's instructions (Qiagen). The PCR products were subsequently cloned into the pCR4Blunt plasmid using the Zero Blunt TOPO® PCR Cloning Kit according to the manufacturer's instructions (Invitrogen) and transformed into DH5-α bacteria (Invitrogen) through standard molecular biology techniques. Plasmid DNA isolated from transformed bacterial clones was sequenced using M13 Forward (5' GTAAAACGACGGCCAGT 3') (SEQ ID NO: 32) and M13 Reverse primers (5' CAGGAAACAGCTATGACC 3') (SEQ ID NO: 33) by Beckman Genomics (Danvers, MA), using standard dideoxy DNA sequencing methods to identify the sequence of the variable

region sequences. The sequences were analyzed using Vector NTI software (Invitrogen) and the IMGT/V-Quest web server to identify and confirm variable region sequences.

[0092] The nucleic acid sequences encoding and the protein sequences defining variable regions of antibody 4B9 are summarized below (amino terminal signal peptide sequences are not shown). CDR sequences (Kabat definition) are shown in bold/underlined in the amino acid sequences.

[0093] Nucleic Acid Sequence Encoding the Heavy Chain Variable Region of Antibody 4B9 (SEQ ID NO: 1)

```
   1 gaggttcagc tccagcagtc tgggactgtg ctggcaaggc ctggggcttc agtgaagatg
  61 tcctgcaaga cttctggcta cacatttacc agctactgga tgcactgggt aaaacagagg
 121 cctggacagg gtctggaatg gataggggct atttatcctg gaaatagtga tactgactac
 181 agccagaagt tcaagggcaa ggccacactg actgcagtca catccgccac cactgcctac
 241 atggaactca gcagcctgac aaatgaggac tctgcggtct attactgttc aaagtttgac
 301 tactggggcc aaggcaccac tctcacagtc tcctca
```

[0094] Protein Sequence Defining the Heavy Chain Variable Region of Antibody 4B9 (SEQ ID NO: 2)

```
   1 evqlqqsgtv larpgasvkm scktsgytft sywmhwvkqr pgqglewiga iypgnsdtdy
  61 sqkfkgkatl tavtsattay melssltned savyycskfd ywgqgttltv ss
```

[0095] Nucleic Acid Sequence Encoding the Kappa Chain Variable Region of Antibody 4B9 (SEQ ID NO: 3)

```
   1 caaattgttc tcacccagtc tccagcactc atgtctgcat ctccagggga gaaggtcacc
  61 atgacctgca gtgccagctc aagtgtaaat tacatgtact ggtaccagca gaagccaaga
 121 tcctccccca aaccctggat ttatctcaca tccaacctgg cttctggagt ccctgctcgc
 181 ttcagtggca gggggtctgg gacctcttac tctctcacaa tcagcagcat ggaggctgaa
 241 gatgctgcca cttattactg ccagcagtgg agtagtaacc cgtacacgtt cggaggggg
 301 accaagctgg aaataaaa
```

[0096] Protein Sequence Defining the Kappa Chain Variable Region of Antibody 4B9 (SEQ ID NO: 4)

```
   1 qivltqspal msaspgekvt mtcsasssvn ymywyqqkpr sspkpwiylt snlasgvpar
  61 fsgrgsgtsy sltissmeae daatyycqqw ssnpytfggg tkleik
```

[0097] Table 1 is a concordance chart showing the SEQ ID NO. of each sequence discussed in this Example.

Table 1

SEQ. ID NO.	Antibody 4B9 Nucleic Acid or Protein
1	Heavy Chain Variable Region—nucleic acid
2	Heavy Chain Variable Region—protein
3	Light (kappa) Chain Variable Region—nucleic acid
4	Light (kappa) Chain Variable Region—protein
5	Heavy Chain CDR_1 (Kabat definition)
6	Heavy Chain CDR_2 (Kabat definition)
11	Heavy Chain CDR_3 (IGMT definition)
12	Light (kappa) Chain CDR_1 (Kabat definition)
13	Light (kappa) Chain CDR_2 (Kabat definition)
14	Light (kappa) Chain CDR_3 (Kabat definition)

[0098] Mouse monoclonal antibody heavy chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 2.

Table 2

	Kabat		
	CDR1	CDR2	CDR3
4B9	SYWMH (SEQ ID NO:5)	AIYPGNSDTDYSQKFKG (SEQ ID NO: 6)	FDY
	Chothia		
	CDR1	CDR2	CDR3
4B9	GYTFTSY (SEQ ID NO: 7)	YPGNSD (SEQ ID NO: 8)	FDY
	IMGT		
	CDR1	CDR2	CDR3
4B9	GYTFTSYW (SEQ ID NO: 9)	IYPGNSDT (SEQ ID NO: 10)	SKFDY (SEQ ID NO: 11)

[0099] Mouse monoclonal antibody Kappa light chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 3.

Table 3

	Kabat/Chothia		
	CDR1	CDR2	CDR3
4B9	SASSSVNYMY (SEQ ID NO: 12)	LTSNLAS (SEQ ID NO: 13)	QQWSSNPYT (SEQ ID NO: 14)
	IMGT		
	CDR1	CDR2	CDR3
4B9	SSVNY (SEQ ID NO: 15)	LTS	QQWSSNPYT (SEQ ID NO: 14)

[0100] To create the complete heavy or kappa chain antibody sequences, each variable sequence above is combined with its respective constant region. For example, a complete heavy chain comprises the heavy variable sequence followed by the murine IgG1 heavy chain

constant sequence and the complete kappa chain comprises a kappa variable sequence followed by the murine kappa light chain constant sequence.

[0101] Nucleic Acid Sequence Encoding the Murine IgG1 Heavy Chain Constant Region (SEQ ID NO: 16)

```
   1 gccaaaacga cacccccatc tgtctatcca ctggcccctg gatctgctgc ccaaactaac
  61 tccatggtga ccctgggatg cctggtcaag ggctatttcc ctgagccagt gacagtgacc
 121 tggaactctg gatccctgtc cagcggtgtg cacaccttcc cagctgtcct gcagtctgac
 181 ctctacactc tgagcagctc agtgactgtc ccctccagca cctggcccag ccagaccgtc
 241 acctgcaacg ttgcccaccc ggccagcagc accaaggtgg acaagaaaat tgtgcccagg
 301 gattgtggtt gtaagccttg catatgtaca gtcccagaag tatcatctgt cttcatcttc
 361 cccccaaagc ccaaggatgt gctcaccatt actctgactc ctaaggtcac gtgtgttgtg
 421 gtagacatca gcaaggatga tcccgaggtc cagttcagct ggtttgtaga tgatgtggag
 481 gtgcacacag ctcagacgca accccgggag gagcagttca acagcacttt ccgctcagtc
 541 agtgaacttc ccatcatgca ccaggactgg ctcaatggca aggagttcaa atgcagggtc
 601 aacagtgcag ctttccctgc ccccatcgag aaaaccatct ccaaaaccaa aggcagaccg
 661 aaggctccac aggtgtacac cattccacct cccaaggagc agatggccaa ggataaagtc
 721 agtctgacct gcatgataac agacttcttc cctgaagaca ttactgtgga gtggcagtgg
 781 aatgggcagc cagcggagaa ctacaagaac actcagccca tcatggacac agatggctct
 841 tacttcgtct acagcaagct caatgtgcag aagagcaact gggaggcagg aaatactttc
 901 acctgctctg tgttacatga gggcctgcac aaccaccata ctgagaagag cctctcccac
 961 tctcctggta aa
```

[0102] Protein Sequence Defining the Murine IgG1 Heavy Chain Constant Region (SEQ ID NO: 17)

```
   1 akttppsvyp lapgsaaqtn smvtlgclvk gyfpepvtvt wnsgslssgv htfpavlqsd
  61 lytlsssvtv psstwpsqtv tcnvahpass tkvdkkivpr dcgckpcict vpevssvfif
 121 ppkpkdvlti tltpkvtcvv vdiskddpev qfswfvddve vhtaqtqpre eqfnstfrsv
 181 selpimhqdw lngkefkcrv nsaafpapie ktisktkgrp kapqvytipp pkeqmakdkv
 241 sltcmitdff peditvewqw ngqpaenykn tqpimdtdgs yfvysklnvq ksnweagntf
 301 tcsvlheglh nhhtekslsh spgk
```

[0103] Nucleic Acid Sequence Encoding the Murine Kappa Light Chain Constant Region (SEQ ID NO: 18)

```
  1 cgggctgatg ctgcaccaac tgtatccatc ttcccaccat ccagtgagca gttaacatct
 61 ggaggtgcct cagtcgtgtg cttcttgaac aacttctacc ccagagacat caatgtcaag
121 tggaagattg atggcagtga acgacaaaat ggtgtcctga acagttggac tgatcaggac
181 agcaaagaca gcacctacag catgagcagc accctcacat tgaccaagga cgagtatgaa
241 cgacataaca gctatacctg tgaggccact cacaagacat caacttcacc cattgtcaag
301 agcttcaaca ggaatgagtg t
```

[0104] Protein Sequence Defining the Murine Kappa Light Chain Constant Region (SEQ ID NO: 19)

```
  1 radaaptvsi fppsseqlts ggasvvcfln nfyprdinvk wkidgserqn gvlnswtdqd
 61 skdstysmss tltltkdeye rhnsytceat hktstspivk sfnrnec
```

[0105] The following sequences represent the actual or contemplated full length heavy and light chain sequences (*i.e.*, containing both the variable and constant regions sequences) for each antibody described in this Example. Signal sequences for proper secretion of the antibodies are also included at the 5' end of the DNA sequences or the amino terminal end of the protein sequences. The variable region sequences can be ligated to other constant region sequences, to produce active full length IgG heavy and light chains.

[0106] Nucleic Acid Sequence Encoding the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgG1 Constant Region) of 4B9 (SEQ ID NO: 20)

```
  1 atggaatgta actggatact tccttttatt ctgtcggtaa cttcaggggt ctactcagag
 61 gttcagctcc agcagtctgg gactgtgctg gcaaggcctg gggcttcagt gaagatgtcc
121 tgcaagactt ctggctacac atttaccagc tactggatgc actgggtaaa acagaggcct
181 ggacagggtc tggaatggat aggggctatt tatcctggaa atagtgatac tgactacagc
241 cagaagttca agggcaaggc cacactgact gcagtcacat ccgccaccac tgcctacatg
301 gaactcagca gcctgacaaa tgaggactct gcggtctatt actgttcaaa gtttgactac
361 tggggccaag gcaccactct cacagtctcc tcagccaaaa cgacacccca atctgtctat
421 ccactggccc ctggatctgc tgcccaaact aactccatgg tgaccctggg atgcctggtc
481 aagggctatt tccctgagcc agtgacagtg acctggaact ctggatccct gtccagcggt
541 gtgcacacct tcccagctgt cctgcagtct gacctctaca ctctgagcag ctcagtgact
601 gtcccctcca gcacctggcc cagccagacc gtcacctgca acgttgccca cccggccagc
661 agcaccaagg tggacaagaa aattgtgccc agggattgtg gttgtaagcc ttgcatatgt
```

```
 721 acagtcccag aagtatcatc tgtcttcatc ttcccccaa agcccaagga tgtgctcacc
 781 attactctga ctcctaaggt cacgtgtgtt gtggtagaca tcagcaagga tgatcccgag
 841 gtccagttca gctggtttgt agatgatgtg gaggtgcaca cagctcagac gcaaccccgg
 901 gaggagcagt tcaacagcac tttccgctca gtcagtgaac ttcccatcat gcaccaggac
 961 tggctcaatg gcaaggagtt caaatgcagg gtcaacagtg cagctttccc tgcccccatc
1021 gagaaaacca tctccaaaac caaaggcaga ccgaaggctc cacaggtgta caccattcca
1081 cctcccaagg agcagatggc caaggataaa gtcagtctga cctgcatgat aacagacttc
1141 ttccctgaag acattactgt ggagtggcag tggaatgggc agccagcgga gaactacaag
1201 aacactcagc ccatcatgga cacagatggc tcttacttcg tctacagcaa gctcaatgtg
1261 cagaagagca actgggaggc aggaaatact ttcacctgct ctgtgttaca tgagggcctg
1321 cacaaccacc atactgagaa gagcctctcc cactctcctg gtaaa
```

[0107] Protein Sequence Defining the Full Length Heavy Chain Sequence (Heavy Chain Variable Region and IgG1 Constant Region) of 4B9 (SEQ ID NO: 21)

```
   1 mecnwilpfi lsvtsgvyse vqlqqsgtvl arpgasvkms cktsgytfts ywmhwvkqrp
  61 gqglewigai ypgnsdtdys qkfkgkatlt avtsattaym elssltneds avyycskfdy
 121 wgqgttltvs sakttppsvy plapgsaaqt nsmvtlgclv kgyfpepvtv twnsgslssg
 181 vhtfpavlqs dlytlsssvt vpsstwpsqt vtcnvahpas stkvdkkivp rdcgckpcic
 241 tvpevssvfi fppkpkdvlt itltpkvtcv vvdiskddpe vqfswfvddv evhtaqtqpr
 301 eeqfnstfrs vselpimhqd wlngkefkcr vnsaafpapi ektisktkgr pkapqvytip
 361 ppkeqmakdk vsltcmitdf fpeditvewq wngqpaenyk ntqpimdtdg syfvysklnv
 421 qksnweagnt ftcsvlhegl hnhhteksls hspgk
```

[0108] Nucleic Acid Sequence Encoding the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 4B9 (SEQ ID NO: 22)

```
   1 atggattttc aagtgcagat tttcagcttc ctgctaatga gtgcctcagt cataatgtcc
  61 aggggacaaa ttgttctcac ccagtctcca gcactcatgt ctgcatctcc aggggagaag
 121 gtcaccatga cctgcagtgc cagctcaagt gtaaattaca tgtactggta ccagcagaag
 181 ccaagatcct cccccaaacc ctggatttat ctcacatcca acctggcttc tggagtccct
 241 gctcgcttca gtggcagggg gtctgggacc tcttactctc tcacaatcag cagcatggag
 301 gctgaagatg ctgccactta ttactgccag cagtggagta gtaacccgta cacgttcgga
 361 gggggggacca agctggaaat aaaacgggct gatgctgcac caactgtatc catcttccca
 421 ccatccagtg agcagttaac atctggaggt gcctcagtcg tgtgcttctt gaacaacttc
 481 taccccagag acatcaatgt caagtggaag attgatggca gtgaacgaca aaatggtgtc
 541 ctgaacagtt ggactgatca ggacagcaaa gacagcacct acagcatgag cagcaccctc
 601 acattgacca aggacgagta tgaacgacat aacagctata cctgtgaggc cactcacaag
 661 acatcaactt cacccattgt caagagcttc aacaggaatg agtgt
```

[0109] Protein Sequence Defining the Full Length Light Chain Sequence (Kappa Chain Variable Region and Constant Region) of 4B9 (SEQ ID NO: 23)

```
  1 mdfqvqifsf llmsasvims rgqivltqsp almsaspgek vtmtcsasss vnymywyqqk
 61 prsspkpwiy ltsnlasgvp arfsgrgsgt sysltissme aedaatyycq qwssnpytfg
121 ggtkleikra daaptvsifp psseqltsgg asvvcflnnf yprdinvkwk idgserqngv
181 lnswtdqdsk dstysmsstl tltkdeyerh nsytceathk tstspivksf nrnec
```

[0110] Table 4 shows the correspondence between the full length sequences of the antibodies discussed in this Example with those presented in the Sequence Listing.

Table 4

SEQ ID NO.	Antibody 4B9 Nucleic Acid or Protein
20	Heavy Variable + IgG1 Constant—nucleic acid
21	Heavy Variable + IgG1 Constant—protein
22	Kappa Variable + Constant—nucleic acid
23	Kappa Variable + Constant—protein

Example 5: Binding Affinities

[0111] The binding affinities and binding kinetics of monoclonal antibody 4B9 were measured with respect to the following proteins (R&D Systems, Inc., Minneapolis, MN):

recombinant human FGFR1 beta (IIIb)/Fc Chimera (rhFGFR1β-IIIc-Fc),

recombinant human FGFR2 beta (IIIb)/Fc Chimera (rhFGFR2β-IIIb-Fc),

recombinant human FGFR2 beta (IIIc)/Fc Chimera (rhFGFR2β-IIIc-Fc),

recombinant human FGFR3 beta (IIIb)/Fc Chimera (rhFGFR3β-IIIb-Fc), and a version of recombinant human FGFR2 beta (IIIb)/Fc (in which the Fc region was removed enzymatically). Binding affinities and binding kinetics were measured by surface plasmon resonance using a Biacore T100 instrument (GE Healthcare, Piscataway, NJ).

[0112] Rabbit anti-mouse IgGs (GE Healthcare) were immobilized on carboxymethylated dextran CM4 sensor chips (GE Healthcare) by amine coupling, using a standard coupling protocol, according to the vendor's instructions (GE Healthcare). The analyses were performed

at 25°C and 37°C, using PBS containing 0.05% surfactant P20 (GE Healthcare) as running buffer.

[0113] The antibodies were captured in individual flow cells at a flow rate of 10 µl/min. Injection time was varied for each antibody to yield an Rmax between 30 and 60 RU. Buffer and FGFR proteins diluted in running buffer were injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 240 seconds at 60 µl/min. The dissociation phase was monitored for up to 900 seconds. The surface was then regenerated with two 60-second injections of 10 mM Glycine-HCl (pH 1.7), at a flow rate of 60 µl/minute. The FGFR protein concentration range tested was 50 to 3.125 nM (two-fold dilutions).

[0114] Kinetic parameters were determined using the kinetic function of the BIAevalutation software (GE Healthcare) with double reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) were determined. Kinetic values of the monoclonal antibodies on FGFR proteins at 25°C and 37°C are summarized in Table 5.

Table 5

Antibody	Target	Temp (°C)	k_a (M^{-1} s^{-1})	k_d (s^{-1})	K_D (M)
4B9	rhFGFR1β-IIIb-Fc	25	no binding	no binding	no binding
4B9	rhFGFR2β-IIIb-Fc	25	9.4E+04	4.6E-05	6.1E-10
4B9	rhFGFR2β-IIIb-Fc	37	3.44E+04	3.16E-05	2.96E-09
4B9	rhFGFR2β-IIIb-cleaved	25	5.5E+04	8.1E-05	4.2E-09
4B9	rhFGFR2β-IIIb-cleaved	37	2.54E+05	2.23E-04	1.20E-09
4B9	rhFGFR2β-IIIc-Fc	25	no binding	no binding	no binding
4B9	rhFGFR3β-IIIb-Fc	25	no binding	no binding	no binding

[0115] The results in Table 5 demonstrate that antibody 4B9 binds rhFGFR2β-IIIb with a K_D of about 5 nM, 4 nM, 3 nM, 2 nM, 1 nM, 750 pM, 650 pM, 610 pM or less. The results also demonstrate that antibody 4B9 does not bind rhFGFR1β-IIIb, rhFGFR2β-IIIc, and rhFGFR3β-IIIb.

Example 6: Anti-Proliferative Activity

[0116] To assess the potency of antibody 4B9 quantitatively, we carried out dose-response studies, using FDCP-1 cells expressing FGFR2-IIIb or FGFR2-IIIc. FDCP-1 cells expressing FGFR2-IIIb or FGFR2-IIIc were seeded in a 96-well plate in the absence of IL3. Varied amounts of FGFs and heparin were added. MTT assays were carried out after 2-3 days. Varied amounts of antibody 4B9-containing supernatants were added to FDCP-1 cells expressing FGFR2-IIIb, FGFR2-IIIc, or C-terminally truncated FGFR2-IIIb, in the presence of FGF1 and heparin. MTT assays were carried out after 2 days. Varied amounts of purified antibody 4B9 were added to FDCP-1 cells expressing FGFR2-IIIb S252W or FGFR2-IIIb N550K in the presence of FGF1 and heparin. MTT assays were carried out after 2 days.

[0117] Antibody 4B9 potently inhibited FGF1-induced proliferation of FDCP-1 cells driven by FGFR2-IIIb, in a dose-dependent manner, while 4B9 had no significant effect on the FGF1-induced proliferation of FDCP cells expressing the FGFR2-IIIc (**FIG. 4**). C-terminally truncated FGFR2-IIIb, which causes constitutive phosphorylation of FRS2 adaptor molecule and activation of downstream signaling, is found in gastric and breast cancer cell lines (Itoh *et al.*, 1994, CANCER RES. 54:3237-3241; Moffa *et al.*, 2004, MOL. CANCER RES. 2:643-652). Antibody 4B9 potently inhibited the proliferation of FDCP-1 cells driven by the C-terminally truncated FGFR2-IIIb (**FIG. 4**).

[0118] FGFR2 mutations have been reported in approximately 12% of endometrial tumor sample (Pollock *et al.*, *supra*; Dutt *et al.*, *supra*). Somatic activating mutations in FGFR2 cluster within the linker region between IgD2 and IgD3, the extracellular juxtamembrane domain, or the kinase domain. Two of the most common mutations in endometrial tumors are the S252W mutation (which alters ligand specificity and increases affinity of ligand binding) and the N550K mutation in the kinase domain (which enhances kinase activity). Purified antibody 4B9 potently inhibited cell proliferation driven by the wild type FGFR2-IIIb, as well as FGFR2-IIIb S252W and FGFR2-IIIb N550K, with IC_{50} values of 0.3 nM, 3.0 nM and 8.1 nM, respectively (**FIG. 5**).

Example 7: Inhibition of FGFR2-Activated Signaling Pathways

[0119] We investigated the effect of antibody 4B9 on FGFR2-activated signaling pathways. To examine the effect of antibody 4B9 on tyrosine phosphorylation of FGFR2, SNU-16 cells were treated with antibodies at a dose of 5 μg/ml for 1 hour at 37°C, followed by stimulation

with heparin alone (20 μg/ml) or heparin-plus-FGF7 (30 ng/ml) for 15 minutes. The cells were lysed in NP-40 lysis buffer containing 1% NP-40, 20 mM Tris-HCl (pH 8.0), 137 mM NaCl, 10% glycerol, 2 mM EDTA and supplemented with protease inhibitors (Roche Applied Science) and Halt phosphatase inhibitors (Thermo Scientific).

[0120] The lysates were analyzed by Western blot with anti-FGFR (Y653/Y654) (R&D Systems, Inc., Minneapolis, MN), anti-FGFR2 (sc-122) (Santa Cruz Biotechnology, Santa Cruz, CA), anti-phospho-ERK1/2 and anti-ERK1/2 (Cell Signaling Technology, Danvers, MA), anti-β-tubulin, clone AA2 (Millipore Corporation; Billerica, MA) antibodies. The immunoblots were detected by chemiluminescent substrate (ECL Plus™, Amersham Pharmacia Biotech, Piscataway, NJ). Human Phospho-RTK and MAPK kinase arrays (R&D systems) were carried out according to manufacturer's instructions (R&D systems). For phospho-RTK arrays, the cells were lysed in NP-40 lysis buffer. The arrays were blocked in Array Buffer 1 at room temperature for one hour prior to the addition of cell lysates diluted in Array Buffer 1 and were then incubated at 4°C overnight. The arrays were visualized by chemiluminescence. For phospho-MAPK arrays, the cells were lysed in Lysis Buffer 6. The diluted cell lysates were added to arrays. After incubation at 4°C overnight, the arrays were mixed with anti-phospho-MAPK antibody for two hours at room temperature and visualized as described above.

[0121] FGF7 induced tyrosine phosphorylation of FGFR2 and subsequent activation of extracellular signal-regulated kinase 1 and 2 (ERK1/2) in Ba/F3 cells overexpressing FGFR2, and in FGFR2-amplifed SNU-16 cells. Antibody 4B9 effectively suppressed the ligand-induced tyrosine phosphorylation of FGFR2 and activation of ERK1/2 in these cells. In addition, antibody 4B9 downregulated the FGFR2 protein level in SNU-16 cells. A slight decrease in the FGFR2 protein level was observed as early as two hours after exposure to the antibody. A dramatic reduction in the protein level was seen at the six-hour time point.

[0122] We investigated activation of downstream signaling pathways in these cell lines, using a phospho-MAPK array, which measures phosphorylation of ERKs, c-Jun NH_2-Terminal Kinases (JNKs), p38 MAPKs, AKTs, and their downstream effector molecules. We found little phosphorylation of ERK1/2 in the absence of ligand stimulation. Stimulation of SNU-16 cells with FGF7 significantly increased the phosphorylation of ERK1/2. We observed an increase in the phosphorylation of mitogen- and stress-activated kinase 2 (MSK2), p38α

MAPK, 90-kD ribosomal protein kinase 1 (RSK1), Akt1, and p70S6 kinase (p70S6K). Antibody 4B9 effectively blocked the phosphorylation of all the downstream signaling proteins activated by FGF7.

Example 8: Inhibition of Tumor Xenograft Growth

[0123] To assess the activity of antibody 4B9 *in vivo*, we tested the effect of antibody 4B9 on the growth of human cancer xenografts harboring amplification of the FGFR2 gene. Out of the four FGFR2-amplified cell lines that were tested, only SNU-16 and MFM-223 yielded tumors in mice. Therefore, we tested the efficacy of antibody 4B9 against SNU-16 and MFM-223 xenograft tumors.

[0124] All mice were treated in accordance with the OLAW Public Health Service Policy on Human Care and Use of Laboratory Animals and the ILAR Guide for the Care and Use of Laboratory Animals. All *in vivo* studies were conducted following the protocols approved by the AVEO Institutional Animal Care and Use Committee. For the SNU-16 *in vivo* studies, 10 week old female C.B-17 SCID mice (Taconic, Germantown, NY) were inoculated subcutaneously into the right flank with $5x10^6$ cells in 1:1 RPMI 1640 (Invitrogen, Carlsbad, CA)/Matrigel (BD Biosciences, San Jose CA). Tumor measurements were taken twice weekly, using vernier calipers. Tumor volume was calculated using the formula: V=0.5 x width x width x length. When tumors approached a volume of 200 mm^3, mice were randomized into five groups of ten animals. The next day, mice were treated with 20 mg/kg mIgG (BioXCell; West Lebanon, NH), 2 mg/kg 4B9, 5 mg/kg 4B9, 10 mg/kg 4B9, or 20 mg/kg 4B9 by intraperitoneal injection. Mice were dosed twice weekly for the duration of the study. Seventy-two hours after the final dose tumor volumes were measured again for calculation of tumor growth inhibition. All statistical analysis was done using GraphPad PRISM® Version 4.00. Final tumor volumes were analyzed using with a one-way analysis of variance and Tukey multiple comparison test.

[0125] SNU-16 xenograft tumors were treated with a control murine IgG at 20 mg/kg or antibody 4B9 at 2, 5, 10 or 20 mg/kg. As shown in **FIG. 6**, each 4B9 treatment group showed significant tumor growth inhibition, as compared to mIgG treated controls (70, 72, 77, and 82%, respectively $p<0.001$) at day 43, which was the last day for the control group to remain in the study. All treatments were well-tolerated with no significant body weight loss. The tumor lysates were also analyzed. Concomitant with inhibition of tyrosine phosphorylation of

FGFR2, antibody 4B9 downregulated the total amount of FGFR2 protein in tumors. No significant difference in the total ERK1/2 or phospho-ERK1/2 was detected in the tumors samples treated with control IgG or 4B9 from tumors collected at the end of study. In contrast to the phospho-receptor tyrosine kinase (RTK) profile of SNU-16 cells *in vitro*, RTK array analysis of SNU-16 xenografts revealed that FGFR2 was the predominant RTK that was tyrosine phosphorylated *in vivo*, and 4B9 significantly inhibited FGFR2 tyrosine phosphorylation in two of the 4B9-treated SNU-16 tumors tested. *In vitro*, the proliferation of SNU-16 cells was not sensitive to the treatment of 4B9. Tyrosine phosphorylation of FGFR2 in SNU-16 cells *in vivo* suggests that the dependence of SNU-16 xenografts on activated FGFR2 signaling *in vivo* explains their sensitivity to treatment with antibody 4B9.

[0126] The effect of antibody 4B9 was also investigated on the *in vivo* growth of FGFR2-amplified breast cancer cell line MFM-223. For these studies, 5-week old female NCr nude mice (Taconic; Germantown, NY) were implanted subcutaneously on the left flank with 0.72 mg 90-day release 17-β estradiol pellets (Innovative Research; Sarasota, FL) and inoculated subcutaneously into the right flank with $10x10^6$ MFM-223 cells in 1:1 EMEM (ATCC; Manassas, VA)/Matrigel. When tumors approached a volume of $200mm^3$, mice were randomized into two groups of ten animals and treated IP with 20 mg/kg mIgG (BioXCell; West Lebanon, NH) or 20 mg/kg 4B9. Mice were dosed twice weekly for the duration of the study. All statistical analysis was done using GraphPad PRISM® Version 4.00. Since there were only two groups in this study final tumor volumes and weights (Day 27, 48 hours after final dose) were analyzed with an unpaired two tailed t-test.

[0127] On day 25, in the MFM-223 xenografts, there was greater than 66% inhibition of tumor volumes (p=0.0015; **FIG. 7**) and final tumor weights (p=0.0188) in 4B9 treated mice, as compared to mIgG-treated controls. All treatments were well-tolerated, with no significant body weight loss. Similar to what was observed in SNU-16 xenografts, 4B9 strongly downregulated the total FGFR2 protein in tumors, concomitant with inhibition of tyrosine phosphorylation of FGFR2. No significant difference in the total or phosphor-ERK1/2 was detected in the tumors samples either treated with the control IgG or 4B9 from tumors collected at the end of study.

Example 9: Humanization of Anti-FGFR2 Antibodies

A. Construction of Humanized FGFR2 Antibodies

[0128] This Example describes the humanization of the murine antibody designated 4B9, and the characterization of the resulting humanized antibodies. The humanized anti-FGFR2 IIIb antibodies were designed using methods well-known in the art. The designed amino acid sequences were converted to codon-optimized DNA sequences and synthesized by DNA2.0, Inc. to include (in the following order): 5' HindIII restriction site, Kozak consensus sequence, amino terminal signal sequence, humanized variable region, human IgG1 or Kappa constant region, stop codon, and a 3' EcoRI restriction site.

[0129] The humanized heavy chains were subcloned into pEE6.4 (Lonza, Basel, Switzerland) via HindIII and EcoRI sites using In-Fusion™ PCR cloning (Clontech, Mountain View, CA). The humanized Kappa light chains were subcloned into pEE14.4 (Lonza) via HindIII and EcoRI sites using In-Fusion™ PCR cloning.

[0130] Humanized antibody chains were transiently transfected into 293T cells to produce antibody. Antibody was purified for subsequent *in vitro* analysis. Binding of the humanized antibodies to human FGFR2 IIIb was measured as described below. The results are summarized in Tables 12 and 13.

[0131] Each of the possible combinations of the humanized immunoglobulin heavy chain and immunoglobulin light chain variable regions are set forth below in Table 6.

Table 6

Light Chain Variable Region	Heavy Chain Variable Region
Hu4B9-65 Kappa (SEQ ID NO: 40)	Hu4B9-65 Heavy (SEQ ID NO: 35)
Hu4B9-65 Kappa (SEQ ID NO: 40)	Hu4B9-82, -83 Heavy (SEQ ID NO: 37)
Hu4B9-82 Kappa (SEQ ID NO: 44)	Hu4B9-65 Heavy (SEQ ID NO: 35)
Hu4B9-82 Kappa (SEQ ID NO: 44)	Hu4B9-82, -83 Heavy (SEQ ID NO: 37)
Hu4B9-83 Kappa (SEQ ID NO: 46)	Hu4B9-65 Heavy (SEQ ID NO: 35)
Hu4B9-83 Kappa (SEQ ID NO: 46)	Hu4B9-82, -83 Heavy (SEQ ID NO: 37)

[0132] The nucleic acid sequences encoding and the protein sequences defining variable regions of the humanized 4B9 antibodies are summarized below (amino terminal signal peptide

sequences are not shown). CDR sequences (Kabat definition) are shown in bold and are underlined in the amino acid sequences.

[0133] Nucleic Acid Sequence Encoding the Hu4B9-65 Heavy Chain Variable Region (SEQ ID NO: 34)

```
   1 caagtgcagc tcgtccaatc gggagccgaa gtgaagaagc ctggttcctc ggtaaaagta
  61 agctgtaagg cgtccggtta cacgtttacc tcatattgga tgcactgggt cagacaggca
 121 cccggacagg gactcgagtg gatgggagcg atctacccgg gcaattcgga cactgattac
 181 agccagaaat tcaaggggag ggtcacgatc acggcagatg agagcacatc aacagcctat
 241 atggagctgt cgtcgcttcg gagcgaggac acggcggtct actactgctc caaattcgac
 301 tattgggggc aggggacctt ggtgaccgtg tcatcc
```

[0134] Protein Sequence Defining the Hu4B9-65 Heavy Chain Variable Region (SEQ ID NO: 35)

```
   1 qvqlvqsgae vkkpgssvkv sckasgytft sywmhwvrqa pgqglewmga iypgnsdtdy
  61 sqkfkgrvti tadeststay melsslrsed tavyycskfd ywgqgtlvtv ss
```

[0135] Nucleic Acid Sequence Encoding the Hu4B9-82, -83 Heavy Chain Variable Region (SEQ ID NO: 36)

```
   1 caagtgcagc tcgtccaatc gggagccgaa gtgaagaagc ctggttcctc ggtaaaagta
  61 agctgtaagg cgtccggtta cacgttttcc tcatattgga tgcactgggt cagacaggca
 121 cccggacagg gactcgagtg gatgggagcg atctacccgg gcaattcgga cactgattac
 181 agccagaaat tccaggggag ggtcacgatc acggcagatg agagcacatc aacagcctat
 241 atggagctgt cgtcgcttcg gagcgaggac acggcggtct actactgctc caaattcgac
 301 tattgggggc aggggacctt ggtgaccgtg tcatcc
```

[0136] Protein Sequence Defining the Hu4B9-82, -83 Heavy Chain Variable Region (SEQ ID NO: 37)

```
   1 qvqlvqsgae vkkpgssvkv sckasgytfs sywmhwvrqa pgqglewmga iypgnsdtdy
  61 sqkfqgrvti tadeststay melsslrsed tavyycskfd ywgqgtlvtv ss
```

[0137] Nucleic Acid Sequence Encoding the Hu4B9-65 Kappa Chain Variable Region (SEQ ID NO: 39)

```
   1 gaaattgtgc tgacccagag cccggcgacc ctgagcctga gcccgggcga acgcgcgacc
```

```
  61 ctgagctgcc gcgcgagcag cagcgtgaac tatatgtatt ggtatcagca gaaaccgggc
 121 caggcgccgc gcccgtggat ttatctgacc agcaaccgcg cgaccggcgt gccggcgcgc
 181 tttagcggca gcggcagcgg caccgattat accctgacca ttagcagcct ggaaccggaa
 241 gattttgcgg tgtattattg ccagcagtgg agcagcaacc cgtatacctt tggccagggc
 301 accaaactgg aaattaaa
```

[0138] Protein Sequence Defining the Hu4B9-65 Kappa Chain Variable Region (SEQ ID NO: 40)

```
   1 eivltqspat lslspgerat lscrasssvn ymywyqqkpg qaprpwiylt snratgvpar
  61 fsgsgsgtdy tltisslepe dfavyycqqw ssnpytfgqg tkleik
```

[0139] Nucleic Acid Sequence Encoding the Hu4B9-82 Kappa Chain Variable Region (SEQ ID NO: 43)

```
   1 gaaatcgtac ttactcagag ccctgccaca ttgtcattgt cacccgggga acgcgccaca
  61 ctgtcgtgcc gggcttcatc gagcgtgaac tacatgtatt ggtatcaaca gaaaccaggc
 121 caagcaccgc gaccttggat ctacttgacg agcaatcgag ccacgggtat ccccgcgagg
 181 ttctccggtt cggggtcggg aactgattac acactgacaa tttcctcgct ggagcccgag
 241 gacttcgcgg tgtactattg tcagcagtgg tcatccaacc cgtacacgtt tggacagggg
 301 acgaagctcg agatcaag
```

[0140] Protein Sequence Defining the Hu4B9-82 Kappa Chain Variable Region (SEQ ID NO: 44)

```
   1 eivltqspat lslspgerat lscrasssvn ymywyqqkpg qaprpwiylt snratgipar
  61 fsgsgsgtdy tltisslepe dfavyycqqw ssnpytfgqg tkleik
```

[0141] Nucleic Acid Sequence Encoding the Hu4B9-83 Kappa Chain Variable Region (SEQ ID NO: 45)

```
   1 gaaatcgtac ttactcagag ccctgccaca ttgtcattgt cacccgggga acgcgccaca
  61 ctgtcgtgcc gggcttcatc gagcgtgaac tacatgtatt ggtatcaaca gaaaccaggc
 121 caagcaccgc gaccttggat ctacttgacg agcaatcgag ccacgggtat ccccgcgagg
 181 ttctccggtt cggggtcggg aactgatttc acactgacaa tttcctcgct ggagcccgag
 241 gacttcgcgg tgtactattg tcagcagtgg tcatccaacc cgtacacgtt tggacagggg
 301 acgaagctcg agatcaag
```

[0142] Protein Sequence Defining the Hu4B9-83 Kappa Chain Variable Region (SEQ ID NO: 46)

```
 1 eivltqspat lslspgerat lscrasssvn ymywyqqkpg qaprpwiylt snratgipar
61 fsgsgsgtdf tltisslepe dfavyycqqw ssnpytfgqg tkleik
```

[0143] The amino acid sequences defining the immunoglobulin heavy chain variable regions for the antibodies produced in Example 9 are aligned in FIG. 8. Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR_1, CDR_2, and CDR_3 (Kabat definition) are identified by boxes (See FIG. 9).

[0144] The amino acid sequences defining the immunoglobulin light chain variable regions for the antibodies in Example 9 are aligned in FIG. 10. Amino terminal signal peptide sequences (for proper expression/secretion) are not shown. CDR_1, CDR_2 and CDR_3 (Kabat definition) are identified by boxes (See FIG. 11).

[0145] Table 7 is a concordance chart showing the SEQ ID NO. of each sequence discussed in this Example.

Table 7

SEQ. ID NO.	Nucleic Acid or Protein
34	Hu4B9-65 Heavy Chain Variable Region—nucleic acid
35	Hu4B9-65 Heavy Chain Variable Region—protein
5	Hu4B9-65 Heavy Chain CDR_1 (Kabat definition)
6	Hu4B9-65 Heavy Chain CDR_2 (Kabat definition)
11	Hu4B9-65 Heavy Chain CDR_3 (IGMT definition)
36	Hu4B9-82, -83 Heavy Chain Variable Region—nucleic acid
37	Hu4B9-82, -83 Heavy Chain Variable Region—protein
5	Hu4B9-82, -83 Heavy Chain CDR_1 (Kabat definition)
38	Hu4B9-82, -83 Heavy Chain CDR_2 (Kabat definition)
11	Hu4B9-82, -83 Heavy Chain CDR_3 (IGMT definition)

SEQ. ID NO.	Nucleic Acid or Protein
39	Hu4B9-65 Light (kappa) Chain Variable Region—nucleic acid
40	Hu4B9-65 Light (kappa) Chain Variable Region—protein
41	Hu4B9-65 Light (kappa) Chain CDR_1 (Kabat definition)
42	Hu4B9-65 Light (kappa) Chain CDR_2 (Kabat definition)
14	Hu4B9-65 Light (kappa) Chain CDR_3 (Kabat definition)
43	Hu4B9-82 Light (kappa) Chain Variable Region—nucleic acid
44	Hu4B9-82 Light (kappa) Chain Variable Region—protein
41	Hu4B9-82 Light (kappa) Chain CDR_1 (Kabat definition)
42	Hu4B9-82 Light (kappa) Chain CDR_2 (Kabat definition)
14	Hu4B9-82 Light (kappa) Chain CDR_3 (Kabat definition)
45	Hu4B9-83 Light (kappa) Chain Variable Region—nucleic acid
46	Hu4B9-83 Light (kappa) Chain Variable Region—protein
41	Hu4B9-83 Light (kappa) Chain CDR_1 (Kabat definition)
42	Hu4B9-83 Light (kappa) Chain CDR_2 (Kabat definition)
14	Hu4B9-83 Light (kappa) Chain CDR_3 (Kabat definition)

[0146] Murine and humanized monoclonal antibody heavy chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 8.

Table 8

	Kabat		
	CDR1	CDR2	CDR3
4B9	SYWMH (SEQ ID NO: 5)	AIYPGNSDTDYSQKFKG (SEQ ID NO: 6)	FDY
Hu4B9-65	SYWMH (SEQ ID NO: 5)	AIYPGNSDTDYSQKFKG (SEQ ID NO: 6)	FDY
Hu4B9-82, -83	SYWMH (SEQ ID NO: 5)	AIYPGNSDTDYSQKFQG (SEQ ID NO: 38)	FDY
	CHOTHIA		
	CDR1	CDR2	CDR3
4B9	GYTFTSY (SEQ ID NO: 7)	YPGNSD (SEQ ID NO: 8)	FDY
Hu4B9-65	GYTFTSY (SEQ ID NO: 7)	YPGNSD (SEQ ID NO: 8)	FDY
Hu4B9-82, -83	GYTFSSY (SEQ ID NO: 47)	YPGNSD (SEQ ID NO: 8)	FDY
	IMGT		
	CDR1	CDR2	CDR3
4B9	GYTFTSYW (SEQ ID NO: 9)	IYPGNSDT (SEQ ID NO: 10)	SKFDY (SEQ ID NO: 11)
Hu4B9-65	GYTFTSYW (SEQ ID NO: 9)	IYPGNSDT (SEQ ID NO: 10)	SKFDY (SEQ ID NO: 11)
Hu4B9-82, -83	GYTFSSYW (SEQ ID NO: 48)	IYPGNSDT (SEQ ID NO: 10)	SKFDY (SEQ ID NO: 11)

[0147] Murine and humanized monoclonal antibody Kappa light chain CDR sequences (Kabat, Chothia, and IMGT definitions) are shown in Table 9.

Table 9

	Kabat/Chothia		
	CDR1	CDR2	CDR3
4B9	SASSSVNYMY (SEQ ID NO: 12)	LTSNLAS (SEQ ID NO: 13)	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-65	RASSSVNYMY (SEQ ID NO: 41)	LTSNRAT (SEQ ID NO: 42)	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-82	RASSSVNYMY (SEQ ID NO: 41)	LTSNRAT (SEQ ID NO: 42)	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-83	RASSSVNYMY (SEQ ID NO: 41)	LTSNRAT (SEQ ID NO: 42)	QQWSSNPYT (SEQ ID NO: 14)
	IGMT		
	CDR1	CDR2	CDR3
4B9	SSVNY (SEQ ID NO: 15)	LTS	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-65	SSVNY (SEQ ID NO: 15)	LTS	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-82	SSVNY (SEQ ID NO: 15)	LTS	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-83	SSVNY (SEQ ID NO: 15)	LTS	QQWSSNPYT (SEQ ID NO: 14)

[0148] To create the complete humanized heavy or kappa chain antibody sequences, each variable sequence above is combined with its respective human constant region. For example, a complete heavy chain comprises a heavy variable sequence followed by a human IgG1 heavy chain constant sequence. A complete kappa chain comprises a kappa variable sequence followed by the human kappa light chain constant sequence.

[0149] Nucleic Acid Sequence Encoding the Human IgG1 Heavy Chain Constant Region (SEQ ID NO: 49)

```
  1 gcctcaacaa aaggaccaag tgtgttccca ctcgccccta gcagcaagag tacatccggg
 61 ggcactgcag cactcggctg cctcgtcaag gattattttc cagagccagt aaccgtgagc
```

```
121 tggaacagtg gagcactcac ttctggtgtc catacttttc ctgctgtcct gcaaagctct
181 ggcctgtact cactcagctc cgtcgtgacc gtgccatctt catctctggg cactcagacc
241 tacatctgta atgtaaacca caagcctagc aatactaagg tcgataagcg ggtggaaccc
301 aagagctgcg acaagactca cacttgtccc ccatgccctg cccctgaact tctgggcggt
361 cccagcgtct ttttgttccc accaaagcct aaagatactc tgatgataag tagaacaccc
421 gaggtgacat gtgttgttgt agacgtttcc cacgaggacc cagaggttaa gttcaactgg
481 tacgttgatg gagtcgaagt acataatgct aagaccaagc ctagagagga gcagtataat
541 agtacatacc gtgtagtcag tgttctcaca gtgctgcacc aagactggct caacggcaaa
601 gaatacaaat gcaaagtgtc caacaaagca ctcccagccc ctatcgagaa gactattagt
661 aaggcaaagg ggcagcctcg tgaaccacag gtgtacactc tgccacccag tagagaggaa
721 atgacaaaga accaagtctc attgacctgc ctggtgaaag gcttctaccc cagcgacatc
781 gccgttgagt gggagagtaa cggtcagcct gagaacaatt acaagacaac ccccccagtg
841 ctggatagtg acgggtcttt ctttctgtac agtaagctga ctgtggacaa gtcccgctgg
901 cagcagggta acgtcttcag ctgttccgtg atgcacgagg cattgcacaa ccactacacc
961 cagaagtcac tgagcctgag cccagggaag
```

[0150] Protein Sequence Defining the Human IgG1 Heavy Chain Constant Region (SEQ ID NO: 50)

```
  1 astkgpsvfp lapsskstsg gtaalgclvk dyfpepvtvs wnsgaltsgv htfpavlqss
 61 glyslssvvt vpssslgtqt yicnvnhkps ntkvdkrvep kscdkthtcp pcpapellgg
121 psvflfppkp kdtlmisrtp evtcvvvdvs hedpevkfnw yvdgvevhna ktkpreeqyn
181 styrvvsvlt vlhqdwlngk eykckvsnka lpapiektis kakgqprepq vytlppsree
241 mtknqvsltc lvkgfypsdi avewesngqp ennykttppv ldsdgsffly skltvdksrw
301 qqgnvfscsv mhealhnhyt qkslslspgk
```

[0151] Nucleic Acid Sequence Encoding the Human Kappa Light Chain Constant Region (SEQ ID NO: 51)

```
  1 cgcacagttg ctgcccccag cgtgttcatt ttcccaccta gcgatgagca gctgaaaagc
 61 ggtactgcct ctgtcgtatg cttgctcaac aacttttacc cacgtgaggc taaggtgcag
121 tggaaagtgg ataatgcact tcaatctgga aacagtcaag agtccgtgac agaacaggac
181 agcaaagact caacttattc actctcttcc accctgactc tgtccaaggc agactatgaa
241 aaacacaagg tatacgcctg cgaggttaca caccagggtt tgtctagtcc tgtcaccaag
301 tccttcaata ggggcgaatg t
```

[0152] Protein Sequence Defining the Human Kappa Light Chain Constant Region (SEQ ID NO: 52)

```
  1 rtvaapsvfi fppsdeqlks gtasvvclln nfypreakvq wkvdnalqsg nsqesvteqd
 61 skdstyslss tltlskadye khkvyacevt hqglsspvtk sfnrgec
```

[0153] The following sequences represent the actual or contemplated full length heavy and light chain sequences (*i.e.*, containing both the variable and constant regions sequences) for each antibody described in this Example. Signal sequences for proper secretion of the antibodies are also included at the 5' end of the DNA sequences or the amino terminal end of the protein sequences. It is also contemplated herein that the variable region sequences can be ligated to other constant region sequences to produce active full length IgG heavy and light chains.

[0154] Nucleic Acid Sequence Encoding the Full Length Humanized Hu4B9-65 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO: 53)

```
   1 atggacatga gagttcctgc tcagctgctc gggttgctgt tgctttggct ccggggtgct
  61 aggtgccaag tgcagctcgt ccaatcggga gccgaagtga agaagcctgg ttcctcggta
 121 aaagtaagct gtaaggcgtc cggttacacg tttacctcat attggatgca ctgggtcaga
 181 caggcacccg gacagggact cgagtggatg ggagcgatct acccgggcaa ttcggacact
 241 gattacagcc agaaattcaa ggggagggtc acgatcacgg cagatgagag cacatcaaca
 301 gcctatatgg agctgtcgtc gcttcggagc gaggacacgg cggtctacta ctgctccaaa
 361 ttcgactatt gggggcaggg gaccttggtg accgtgtcat ccgcctcaac aaaaggacca
 421 agtgtgttcc cactcgcccc tagcagcaag agtacatccg ggggcactgc agcactcggc
 481 tgcctcgtca aggattattt tccagagcca gtaaccgtga gctggaacag tggagcactc
 541 acttctggtg tccatacttt tcctgctgtc ctgcaaagct ctggcctgta ctcactcagc
 601 tccgtcgtga ccgtgccatc ttcatctctg ggcactcaga cctacatctg taatgtaaac
 661 cacaagccta gcaatactaa ggtcgataag cgggtggaac ccaagagctg cgacaagact
 721 cacacttgtc ccccatgccc tgcccctgaa cttctgggcg gtcccagcgt ctttttgttc
 781 ccaccaaagc ctaaagatac tctgatgata agtagaacac ccgaggtgac atgtgttgtt
 841 gtagacgttt cccacgagga cccagaggtt aagttcaact ggtacgttga tggagtcgaa
 901 gtacataatg ctaagaccaa gcctagagag gagcagtata atagtacata ccgtgtagtc
 961 agtgttctca cagtgctgca ccaagactgg ctcaacggca aagaatacaa atgcaaagtg
1021 tccaacaaag cactcccagc ccctatcgag aagactatta gtaaggcaaa ggggcagcct
1081 cgtgaaccac aggtgtacac tctgccaccc agtagagagg aaatgacaaa gaaccaagtc
1141 tcattgacct gcctggtgaa aggcttctac cccagcgaca tcgccgttga gtgggagagt
```

```
1201 aacggtcagc ctgagaacaa ttacaagaca acccccccag tgctggatag tgacgggtct
1261 ttctttctgt acagtaagct gactgtggac aagtcccgct ggcagcaggg taacgtcttc
1321 agctgttccg tgatgcacga ggcattgcac aaccactaca cccagaagtc actgagcctg
1381 agcccaggga ag
```

[0155] Protein Sequence Defining the Full Length Humanized Hu4B9-65 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO: 54)

```
   1 mdmrvpaqll glllwlrga rcqvqlvqsg aevkkpgssv kvsckasgyt ftsywmhwvr
  61 qapgqglewm gaiypgnsdt dysqkfkgrv titadestst aymelsslrs edtavyycsk
 121 fdywgqgtlv tvssastkgp svfplapssk stsggtaalg clvkdyfpep vtvswnsgal
 181 tsgvhtfpav lqssglysls svvtvpsssl gtqtyicnvn hkpsntkvdk rvepkscdkt
 241 htcppcpape llggpsvflf ppkpkdtlmi srtpevtcvv vdvshedpev kfnwyvdgve
 301 vhnaktkpre eqynstyrvv svltvlhqdw lngkeykckv snkalpapie ktiskakgqp
 361 repqvytlpp sreemtknqv sltclvkgfy psdiavewes ngqpennykt tppvldsdgs
 421 fflyskltvd ksrwqqgnvf scsvmhealh nhytqkslsl spgk
```

[0156] Nucleic Acid Sequence Encoding the Full Length Humanized Hu4B9-82, -83 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO: 55)

```
   1 atggacatga gagttcctgc tcagctgctc gggttgctgt tgctttggct ccggggtgct
  61 aggtgccaag tgcagctcgt ccaatcggga gccgaagtga agaagcctgg ttcctcggta
 121 aaagtaagct gtaaggcgtc cggttacacg ttttcctcat attggatgca ctgggtcaga
 181 caggcacccg gacagggact cgagtggatg ggagcgatct acccgggcaa ttcggacact
 241 gattacagcc agaaattcca ggggagggtc acgatcacgg cagatgagag cacatcaaca
 301 gcctatatgg agctgtcgtc gcttcggagc gaggacacgg cggtctacta ctgctccaaa
 361 ttcgactatt gggggcaggg gaccttggtg accgtgtcat ccgcctcaac aaaaggacca
 421 agtgtgttcc cactcgcccc tagcagcaag agtacatccg ggggcactgc agcactcggc
 481 tgcctcgtca aggattattt tccagagcca gtaaccgtga gctggaacag tggagcactc
 541 acttctggtg tccatacttt tcctgctgtc ctgcaaagct ctggcctgta ctcactcagc
 601 tccgtcgtga ccgtgccatc ttcatctctg ggcactcaga cctacatctg taatgtaaac
 661 cacaagccta gcaatactaa ggtcgataag cgggtggaac ccaagagctg cgacaagact
 721 cacacttgtc ccccatgccc tgcccctgaa cttctgggcg gtcccagcgt ctttttgttc
 781 ccaccaaagc ctaaagatac tctgatgata agtagaacac ccgaggtgac atgtgttgtt
 841 gtagacgttt cccacgagga cccagaggtt aagttcaact ggtacgttga tggagtcgaa
 901 gtacataatg ctaagaccaa gcctagagag gagcagtata atagtacata ccgtgtagtc
```

```
 961 agtgttctca cagtgctgca ccaagactgg ctcaacggca aagaatacaa atgcaaagtg
1021 tccaacaaag cactcccagc ccctatcgag aagactatta gtaaggcaaa ggggcagcct
1081 cgtgaaccac aggtgtacac tctgccaccc agtagagagg aaatgacaaa gaaccaagtc
1141 tcattgacct gcctggtgaa aggcttctac cccagcgaca tcgccgttga gtgggagagt
1201 aacggtcagc ctgagaacaa ttacaagaca acccccccag tgctggatag tgacgggtct
1261 ttctttctgt acagtaagct gactgtggac aagtcccgct ggcagcaggg taacgtcttc
1321 agctgttccg tgatgcacga ggcattgcac aaccactaca cccagaagtc actgagcctg
1381 agcccaggga ag
```

[0157] Protein Sequence Defining the Full Length Humanized Hu4B9-82, -83 Heavy Chain (Humanized Heavy Chain Variable Region and Human IgG1 Constant Region) (SEQ ID NO: 56)

```
   1 mdmrvpaqll glllwlrga rcqvqlvqsg aevkkpgssv kvsckasgyt fssywmhwvr
  61 qapgqglewm gaiypgnsdt dysqkfqgrv titadestst aymelsslrs edtavyycsk
 121 fdywgqgtlv tvssastkgp svfplapssk stsggtaalg clvkdyfpep vtvswnsgal
 181 tsgvhtfpav lqssglysls svvtvpsssl gtqtyicnvn hkpsntkvdk rvepkscdkt
 241 htcppcpape llggpsvflf ppkpkdtlmi srtpevtcvv vdvshedpev kfnwyvdgve
 301 vhnaktkpre eqynstyrvv svltvlhqdw lngkeykckv snkalpapie ktiskakgqp
 361 repqvytlpp sreemtknqv sltclvkgfy psdiavewes ngqpennykt tppvldsdgs
 421 fflyskltvd ksrwqqgnvf scsvmhealh nhytqkslsl spgk
```

[0158] Nucleic Acid Sequence Encoding the Full Length Humanized Hu4B9-65 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 57)

```
   1 atggacatga gggtgcccgc tcaactgctg gggctgctgc tgctgtggct gagaggagct
  61 cgttgcgaaa ttgtgctgac ccagagcccg gcgaccctga gcctgagccc gggcgaacgc
 121 gcgaccctga gctgccgcgc gagcagcagc gtgaactata tgtattggta tcagcagaaa
 181 ccgggccagg cgccgcgccc gtggatttat ctgaccagca accgcgcgac cggcgtgccg
 241 gcgcgcttta gcggcagcgg cagcggcacc gattataccc tgaccattag cagcctggaa
 301 ccggaagatt ttgcggtgta ttattgccag cagtggagca gcaacccgta tacctttggc
 361 cagggcacca aactggaaat taaacgcaca gttgctgccc ccagcgtgtt cattttccca
 421 cctagcgatg agcagctgaa aagcggtact gcctctgtcg tatgcttgct caacaacttt
 481 tacccacgtg aggctaaggt gcagtggaaa gtggataatg cacttcaatc tggaaacagt
 541 caagagtccg tgacagaaca ggacagcaaa gactcaactt attcactctc ttccaccctg
 601 actctgtcca aggcagacta tgaaaaacac aaggtatacg cctgcgaggt tacacaccag
 661 ggtttgtcta gtcctgtcac caagtccttc aataggggcg aatgt
```

[0159] Protein Sequence Defining the Full Length Humanized Hu4B9-65 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 58)

```
  1 mdmrvpaqll glllwlrga rceivltqsp atlslspger atlscrasss vnymywyqqk
 61 pgqaprpwiy ltsnratgvp arfsgsgsgt dytltissle pedfavyycq qwssnpytfg
121 qgtkleikrt vaapsvfifp psdeqlksgt asvvcllnnf ypreakvqwk vdnalqsgns
181 qesvteqdsk dstyslsstl tlskadyekh kvyacevthq glsspvtksf nrgec
```

[0160] Nucleic Acid Sequence Encoding the Full Length Humanized Hu4B9-82 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 59)

```
  1 atggacatga gggtgcccgc tcaactgctg gggctgctgc tgctgtggct gagaggagct
 61 cgttgcgaaa tcgtacttac tcagagccct gccacattgt cattgtcacc cggggaacgc
121 gccacactgt cgtgccgggc ttcatcgagc gtgaactaca tgtattggta tcaacagaaa
181 ccaggccaag caccgcgacc ttggatctac ttgacgagca atcgagccac gggtatcccc
241 gcgaggttct ccggttcggg gtcgggaact gattacacac tgacaatttc ctcgctggag
301 cccgaggact tcgcggtgta ctattgtcag cagtggtcat ccaacccgta cacgtttgga
361 caggggacga agctcgagat caagcgcaca gttgctgccc ccagcgtgtt cattttccca
421 cctagcgatg agcagctgaa aagcggtact gcctctgtcg tatgcttgct caacaacttt
481 tacccacgtg aggctaaggt gcagtggaaa gtggataatg cacttcaatc tggaaacagt
541 caagagtccg tgacagaaca ggacagcaaa gactcaactt attcactctc ttccaccctg
601 actctgtcca aggcagacta tgaaaaacac aaggtatacg cctgcgaggt tacacaccag
661 ggtttgtcta gtcctgtcac caagtccttc aataggggcg aatgt
```

[0161] Protein Sequence Defining the Full Length Humanized Hu4B9-82 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 60)

```
  1 mdmrvpaqll glllwlrga rceivltqsp atlslspger atlscrasss vnymywyqqk
 61 pgqaprpwiy ltsnratgip arfsgsgsgt dytltissle pedfavyycq qwssnpytfg
121 qgtkleikrt vaapsvfifp psdeqlksgt asvvcllnnf ypreakvqwk vdnalqsgns
181 qesvteqdsk dstyslsstl tlskadyekh kvyacevthq glsspvtksf nrgec
```

[0162] Nucleic Acid Sequence Encoding the Full Length Humanized Hu4B9-83 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 61)

```
   1 atggacatga gggtgcccgc tcaactgctg gggctgctgc tgctgtggct gagaggagct
  61 cgttgcgaaa tcgtacttac tcagagccct gccacattgt cattgtcacc cggggaacgc
 121 gccacactgt cgtgccgggc ttcatcgagc gtgaactaca tgtattggta tcaacagaaa
 181 ccaggccaag caccgcgacc ttggatctac ttgacgagca atcgagccac gggtatcccc
 241 gcgaggttct ccggttcggg gtcgggaact gatttcacac tgacaatttc ctcgctggag
 301 cccgaggact tcgcggtgta ctattgtcag cagtggtcat ccaacccgta cacgtttgga
 361 caggggacga agctcgagat caagcgcaca gttgctgccc ccagcgtgtt cattttccca
 421 cctagcgatg agcagctgaa aagcggtact gcctctgtcg tatgcttgct caacaacttt
 481 tacccacgtg aggctaaggt gcagtggaaa gtggataatg cacttcaatc tggaaacagt
 541 caagagtccg tgacagaaca ggacagcaaa gactcaactt attcactctc ttccaccctg
 601 actctgtcca aggcagacta tgaaaaacac aaggtatacg cctgcgaggt tacacaccag
 661 ggtttgtcta gtcctgtcac caagtccttc aataggggcg aatgt
```

[0163] Protein Sequence Defining the Full Length Humanized Hu4B9-83 Light Chain (Humanized Kappa Chain Variable Region and Human Constant Region) (SEQ ID NO: 62)

```
   1 mdmrvpaqll glllwlrga rceivltqsp atlslspger atlscrasss vnymywyqqk
  61 pgqaprpwiy ltsnratgip arfsgsgsgt dftltissle pedfavyycq qwssnpytfg
 121 qgtkleikrt vaapsvfifp psdeqlksgt asvvcllnnf ypreakvqwk vdnalqsgns
 181 qesvteqdsk dstyslsstl tlskadyekh kvyacevthq glsspvtksf nrgec
```

[0164] For convenience, Table 10 provides a concordance chart showing the SEQ ID NO. of each sequence discussed in this Example.

Table 10

SEQ ID NO.	Nucleic Acid or Protein
49	Human IgG1 constant—nucleic acid
50	Human IgG1 constant—protein
51	Human Kappa constant—nucleic acid
52	Human Kappa constant—protein
53	Humanized Hu4B9-65 Heavy Human Variable + Human IgG1 constant—nucleic acid
54	Humanized Hu4B9-65 Heavy Human Variable + Human IgG1 constant—protein
55	Humanized Hu4B9-82, -83 Heavy Human Variable + Human IgG1 constant—nucleic acid
56	Humanized Hu4B9-82,-83 Heavy Human Variable + Human IgG1 constant—protein
57	Humanized Hu4B9-65 Human Variable + Human Kappa constant—nucleic acid
58	Humanized Hu4B9-65 Human Variable + Human Kappa constant—protein
59	Humanized Hu4B9-82 Human Variable + Human Kappa constant—nucleic acid
60	Humanized Hu4B9-82 Human Variable + Human Kappa constant—protein
61	Humanized Hu4B9-83 Human Variable + Human Kappa constant—nucleic acid
62	Humanized Hu4B9-83 Human Variable + Human Kappa constant—protein

[0165] Table 11 below shows antibodies containing each of the possible combinations of the full-length humanized immunoglobulin heavy and light chains.

Table 11

Antibody Name	Light Chain	Heavy Chain
Hu4B9-65	Hu4B9-65 Kappa (SEQ ID NO: 58)	Hu4B9-65 Heavy (SEQ ID NO: 54)
Hu4B9-84	Hu4B9-65 Kappa (SEQ ID NO: 58)	Hu4B9-82, -83 Heavy (SEQ ID NO: 56)
Hu4B9-85	Hu4B9-82 Kappa (SEQ ID NO: 60)	Hu4B9-65 Heavy (SEQ ID NO: 54)
Hu4B9-82	Hu4B9-82 Kappa (SEQ ID NO: 60)	Hu4B9-82, -83 Heavy (SEQ ID NO: 56)
Hu4B9-86	Hu4B9-83 Kappa (SEQ ID NO: 62)	Hu4B9-65 Heavy (SEQ ID NO: 54)
Hu4B9-83	Hu4B9-83 Kappa (SEQ ID NO: 62)	Hu4B9-82, -83 Heavy (SEQ ID NO: 56)

[0166] Three of the possible antibody constructs containing the full length immunoglobulin heavy and light chains containing humanized variable regions are designated below:

Hu4B9-65 = Humanized Hu4B9-65 Heavy Chain Variable Region and Human IgG1 Constant Region (SEQ ID NO: 54) plus Hu4B9-65 Light Chain Variable Region and Human Kappa Constant Region (SEQ ID NO: 58)

Hu4B9-82 = Humanized Hu4B9-82, -83 Heavy Chain Variable Region and Human IgG1 Constant Region (SEQ ID NO: 56) plus Hu4B9-82 Light Chain Variable Region and Human Kappa Constant Region (SEQ ID NO: 60)

Hu4B9-83 = Humanized Hu4B9-82, -83 Heavy Chain Variable Region and Human IgG1 Constant Region (SEQ ID NO: 56) plus Hu4B9-83 Light Chain Variable Region and Human Kappa Constant Region (SEQ ID NO: 62)

B. Binding Affinities of Humanized Anti-FGFR2 Monoclonal Antibodies

[0167] The binding affinities and kinetics of interaction of monoclonal antibodies produced in Example 9 against monomeric recombinant human FGFR2 beta IIIb (rhFGFR2β-IIIb-cleaved) were measured by surface plasmon resonance using a Biacore T100 (Biacore (GE Healthcare), Piscataway, NJ) instrument.

[0168] Goat anti-human IgG Fc (Jackson ImmunoResearch, Catalog No. 109-005-098) was immobilized on carboxymethylated dextran CM4 sensor chips (Biacore) by amine coupling (Biacore) using a standard coupling protocol according to the vendor's instructions. The analyses were performed at 25°C and 37°C using PBS (Invitrogen) containing 0.05% surfactant P20 (Biacore) as running buffer.

[0169] Purified antibodies were captured in individual flow cells at a flow rate of 10 µl/minute. Injection time was varied for each antibody to yield an R_{max} between 30 and 90 RU. Buffer or rhFGFR2β-IIIb-cleaved diluted in running buffer was injected sequentially over a reference surface (no antibody captured) and the active surface (antibody to be tested) for 240 seconds at 60 µl/minute. The dissociation phase was monitored for up to 900 seconds. The surface was then regenerated with two 60 second injections of glycine pH 2.25 (made from glycine pH 2.0 (Biacore) and pH 2.5 (Biacore)) at 30 µl/minute. Experiments were conducted using concentrations of rhFGFR2β-IIIb-cleaved between 20 and 1.25 nM (a two-fold serial dilution).

[0170] Kinetic parameters were determined using the kinetic function of the BIAevaluation software (Biacore) with double reference subtraction. Kinetic parameters for each antibody, k_a (association rate constant), k_d (dissociation rate constant) and K_D (equilibrium dissociation constant) were determined. The kinetic values of certain purified monoclonal antibodies (*i.e.*, Hu4B9-65, Hu4B9-82, and Hu4B9-83) on rhFGFR2β-IIIb-cleaved at 25°C are summarized in Table 12.

Table 12

Antibody	ka (1/Ms)	kd (1/s)	KD (M)	n
hu4B9-65	2.4E+05	6.5E-05	2.6E-10	4
hu4B9-82	1.9E+05	9.4E-05	4.9E-10	2
hu4B9-83	2.6E+05	8.9E-05	3.5E-10	3

[0171] The results in Table 12 demonstrate the purified antibodies have affinities ranging from about 260 pM to about 490 pM when tested at 25°C.

[0172] The kinetic values of certain purified monoclonal antibodies (*i.e.*, Hu4B9-65, Hu4B9-82, and Hu4B9-83) on rhFGFR2β-IIIb-cleaved at 37°C are summarized in Table 13.

Table 13

Antibody	ka (1/Ms)	kd (1/s)	KD (M)	n
hu4B9-65	3.7E+05	2.8E-04	8.9E-10	7
hu4B9-82	4.0E+05	3.6E-04	9.3E-10	3
hu4B9-83	3.2E+05	2.9E-04	9.2E-10	3

[0173] The results in Table 13 demonstrate the purified antibodies have affinities ranging from about 890 pM to about 930 pM when tested at 37°C.

Example 10: Anti-Proliferative Activity of Humanized Anti-FGFR2 Monoclonal Antibodies

[0174] The potency of humanized anti-FGFR2 antibodies was assessed in a cell-based proliferation assay. FDCP-1 cells expressing FGFR2-IIIb were seeded in a 96-well plate in IL-3 free medium containing 8 ng/ml of FGF1 and 5 μg/ml of heparin. Serial dilutions of the antibodies were prepared and added to the plate. After two days of incubation, cell proliferation was examined by a MTT assay as described above in Example 1.

[0175] As shown in **FIG. 12**, humanized antibodies (Hu4B9-65, Hu4B9-82, and Hu4B9-83) demonstrated dose-dependent inhibition of FGF1-induced FDCP-FGFR2-IIIb cell proliferation. The average IC50s of the 4B9, Hu4B9-65, Hu4B9-82 and Hu4B9-83 from three independent experiments are 1.4, 4.9, 5.7 and 4.7 nM, respectively.

INCORPORATION BY REFERENCE

[0176] The entire disclosure of each of the patent documents and scientific articles referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[0177] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and the range of equivalency of the claims are intended to be embraced therein.

[0178] **WHAT IS CLAIMED IS:**

1. An isolated antibody that binds human FGFR2 comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region selected from the group consisting of:

(a) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (**4B9; Hu4B9-65; Hu4B9-82, -83**) and SEQ ID NO: 7 (**4B9; Hu4B9-65**), a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6 (**4B9; Hu4B9-65**), and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 11 (**4B9; Hu4B9-65; Hu4B9-82, -83**); and

(ii) an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 12 (**4B9**), a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 13 (**4B9**), and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 14 (**4B9; Hu4B9-65; Hu4B9-82; Hu4B9-83**);

(b) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (**4B9; Hu4B9-65; Hu4B9-82, -83**) and SEQ ID NO: 7 (**4B9; Hu4B9-65**), a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 6 (**4B9; Hu4B9-65**), and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 11 (**4B9; Hu4B9-65; Hu4B9-82, -83**); and

(ii) an immunoglobulin light chain variable region comprising a CDR_{L1} the amino acid sequence of SEQ ID NO: 41 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**), a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 42 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**), and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 14 (**4B9; Hu4B9-65; Hu4B9-82; Hu4B9-83**); and

(c) (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 5 (**4B9; Hu4B9-65; Hu4B9-82, -83**) and SEQ ID NO: 47 (**Hu4B9-82, -83**), a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 38 (**Hu4B9-82, -83**), and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 11 (**4B9; Hu4B9-65; Hu4B9-82, -83**); and

(ii) an immunoglobulin light chain variable region comprising a CDR_{L1} the amino acid sequence of SEQ ID NO: 41 (**Hu4B9-65; Hu4B9-82; Hu4B9-83**), a CDR_{L2} comprising the

amino acid sequence of SEQ ID NO: 42 **(Hu4B9-65; Hu4B9-82; Hu4B9-83)**, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 14 **(4B9; Hu4B9-65; Hu4B9-82; Hu4B9-83)**.

2. The antibody of claim 1, wherein the CDR sequences are interposed between human and humanized framework sequences.

3. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain variable region of claim 1.

4. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain variable region of claim 1.

5. An expression vector containing the nucleic acid of claim 3.

6. An expression vector containing the nucleic acid of claim 4.

7. The expression vector of claim 6, further comprising the nucleic acid of claim 5.

8. A host cell comprising the expression vector of claim 5.

9. A host cell comprising the expression vector of claim 6.

10. A host cell comprising the expression vector of claim 7.

11. The host cell of claim 9, further comprising the expression vector of claim 5.

12. A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region, the method comprising:

(a) growing the host cell of claim 8 or 9 under conditions so that the host cell express the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region; and

(b) purifying the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region.

13. A method of producing an antibody that binds human FGFR2 or an antigen binding fragment of the antibody, the method comprising:

(a) growing the host cell of claim 10 or 11 under conditions so that the host cell expresses a polypeptide comprising the immunoglobulin heavy chain variable region and/or the

immunoglobulin light chain variable region, thereby producing the antibody or the antigen-binding fragment of the antibody; and

(b) purifying the antibody or the antigen-binding fragment of the antibody.

14. An isolated antibody that binds human FGFR2, comprising an immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region selected from the group consisting of:

(a) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 2 **(4B9)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 4 **(4B9)**;

(b) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 35 **(Hu4B9-65)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 40 **(Hu4B9-65)**;

(c) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 **(Hu4B9-82, -83)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 44 **(Hu4B9-82)**; and

(d) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 37 **(Hu4B9-82, -83)**, and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 46 **(Hu4B9-83)**.

15. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain variable region of claim 14.

16. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain variable region of claim 14.

17. An expression vector containing the nucleic acid of claim 15.

18. An expression vector containing the nucleic acid of claim 16.

19. The expression vector of claim 18, further comprising the nucleic acid of claim 15.

20. A host cell comprising the expression vector of claim 17.

21. A host cell comprising the expression vector of claim 18.

22. A host cell comprising the expression vector of claim 19.

23. The host cell of claim 21, further comprising the expression vector of claim 17.

24. A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region, the method comprising:

(a) growing the host cell of claim 20 or 21 under conditions so that the host cell express the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region; and

(b) purifying the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region.

25. A method of producing an antibody that binds human FGFR2 or an antigen binding fragment of the antibody, the method comprising:

(a) growing the host cell of claim 22 or 23 under conditions so that the host cell expresses a polypeptide comprising the immunoglobulin heavy chain variable region and/or the immunoglobulin light chain variable region, thereby producing the antibody or the antigen-binding fragment of the antibody; and

(b) purifying the antibody or the antigen-binding fragment of the antibody.

26. An isolated antibody that binds human FGFR2 comprising an immunoglobulin heavy chain and an immunoglobulin light chain selected from the group consisting of:

(a) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 21 **(4B9)**, and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 23 **(4B9)**;

(b) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 54 **(Hu4B9-65)**, and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 58 **(Hu4B9-65)**;

(c) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 56 **(Hu4B9-82, -83)**, and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 60 **(Hu4B9-82)**; and

(d) an immunoglobulin heavy chain comprising the amino acid sequence of SEQ ID NO: 56 **(Hu4B9-82, -83)**, and an immunoglobulin light chain comprising the amino acid sequence of SEQ ID NO: 62 **(Hu4B9-83)**.

27. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain of claim 26.

28. An isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain of claim 26.

29. An expression vector containing the nucleic acid of claim 27.

30. An expression vector containing the nucleic acid of claim 28.

31. The expression vector of claim 30, further comprising the nucleic acid of claim 27.

32. A host cell comprising the expression vector of claim 29.

33. A host cell comprising the expression vector of claim 30.

34. A host cell comprising the expression vector of claim 31.

35. The host cell of claim 33, further comprising the expression vector of claim 29.

36. A method of producing a polypeptide comprising an immunoglobulin heavy chain variable region or an immunoglobulin light chain variable region, the method comprising:

(a) growing the host cell of claim 32 or 33 under conditions so that the host cell express the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region; and

(b) purifying the polypeptide comprising the immunoglobulin heavy chain variable region or the immunoglobulin light chain variable region.

37. A method of producing an antibody that binds human FGFR2 or an antigen binding fragment of the antibody, the method comprising:

(a) growing the host cell of claim 34 or 35 under conditions so that the host cell expresses a polypeptide comprising the immunoglobulin heavy chain variable region and/or the immunoglobulin light chain variable region, thereby producing the antibody or the antigen-binding fragment of the antibody; and

(b) purifying the antibody or the antigen-binding fragment of the antibody.

38. The antibody of claim 1, 14, or 26, wherein the antibody has a K_D of 500 pM or lower as measured by surface plasmon resonance.

39. A method of inhibiting or reducing proliferation of a tumor cell comprising exposing the cell to an effective amount of the antibody of claim 1, 14, or 26 to inhibit or reduce proliferation of the tumor cell.

40. A method of inhibiting or reducing tumor growth in a mammal, the method comprising exposing the mammal to an effective amount of the antibody of claim 1, 14, or 26 to inhibit or reduce proliferation of the tumor.

41. A method of treating cancer in a mammal, the method comprising administering an effective amount of the antibody of claim 1, 14, or 26 to a mammal in need thereof.

42 The method of claim 41, wherein the cancer is selected from the group consisting of breast, ovarian, prostate, cervical, colorectal, lung, pancreatic, gastric, and head and neck cancer.

43. The method of claim 41, wherein the mammal is a human.

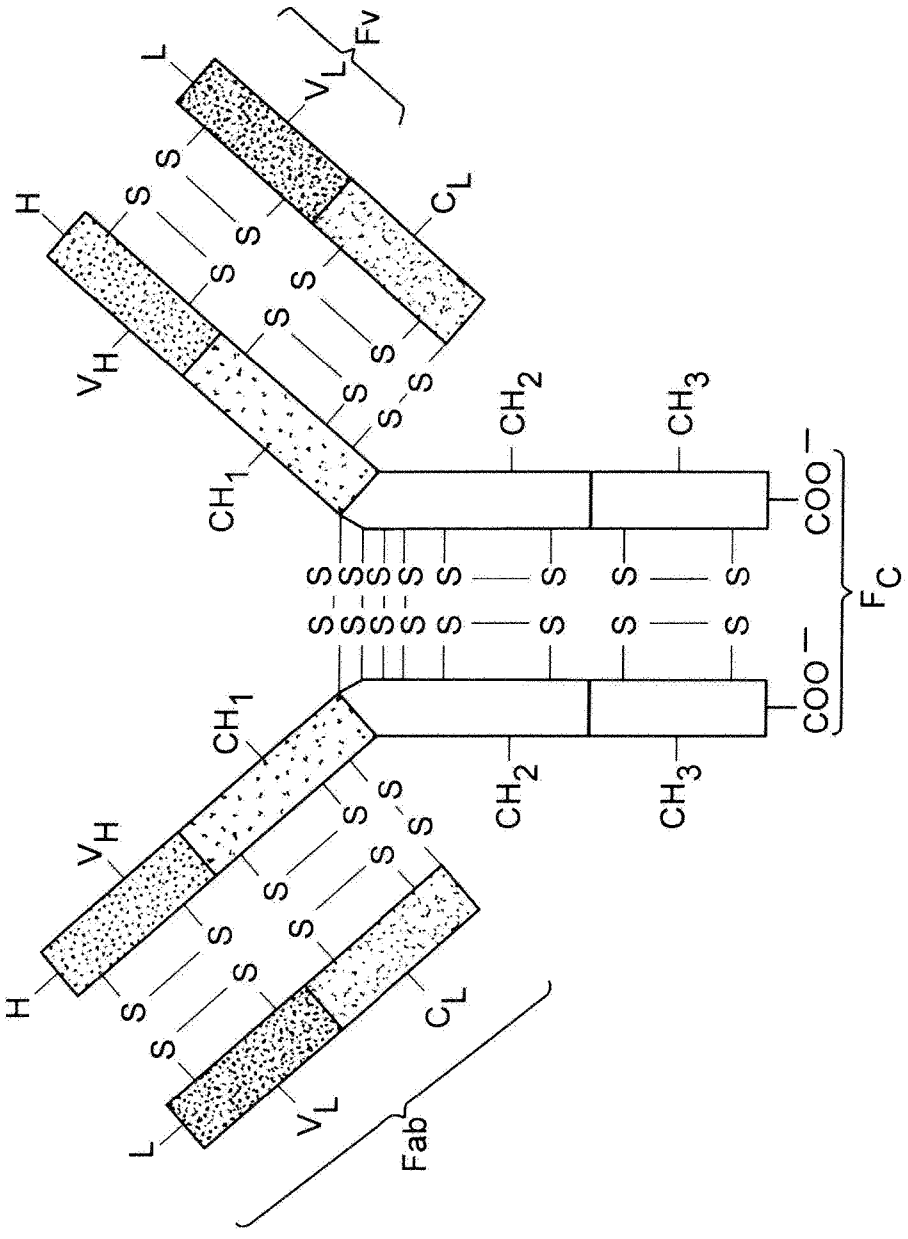


FIG. 1

FIG. 2

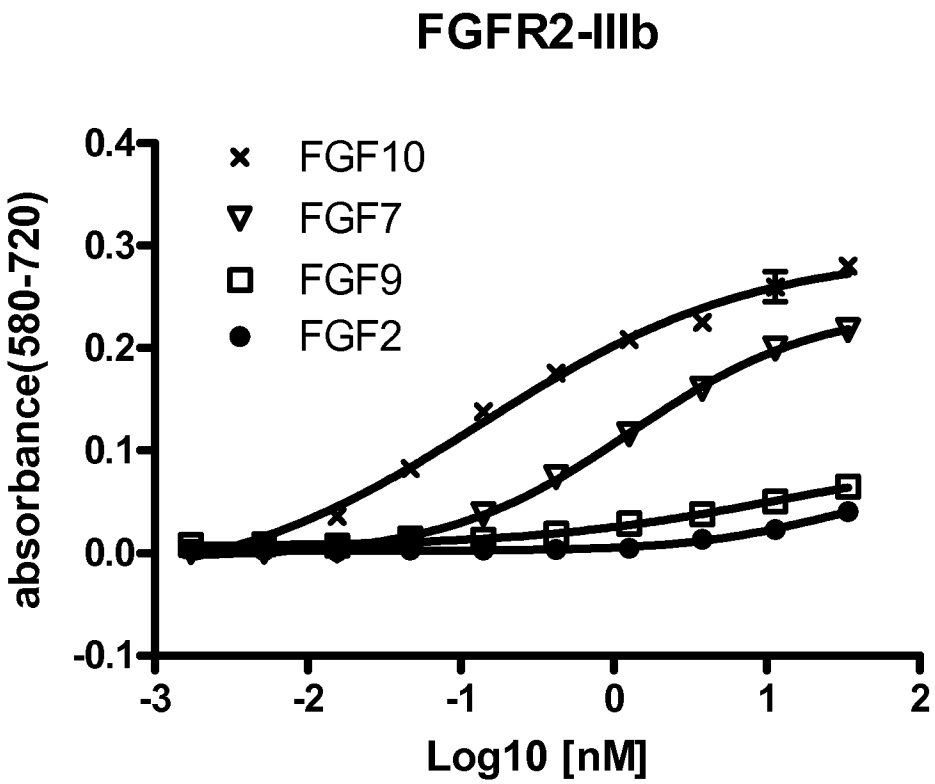

FGFR2-IIIb
FGF10
FGF7
FGF9
FGF2
absorbance(580-720)
0.4
0.3
0.2
0.1
0.0
-0.1
-3
-2
-1
0
1
2
Log10 [nM]

FIG. 3

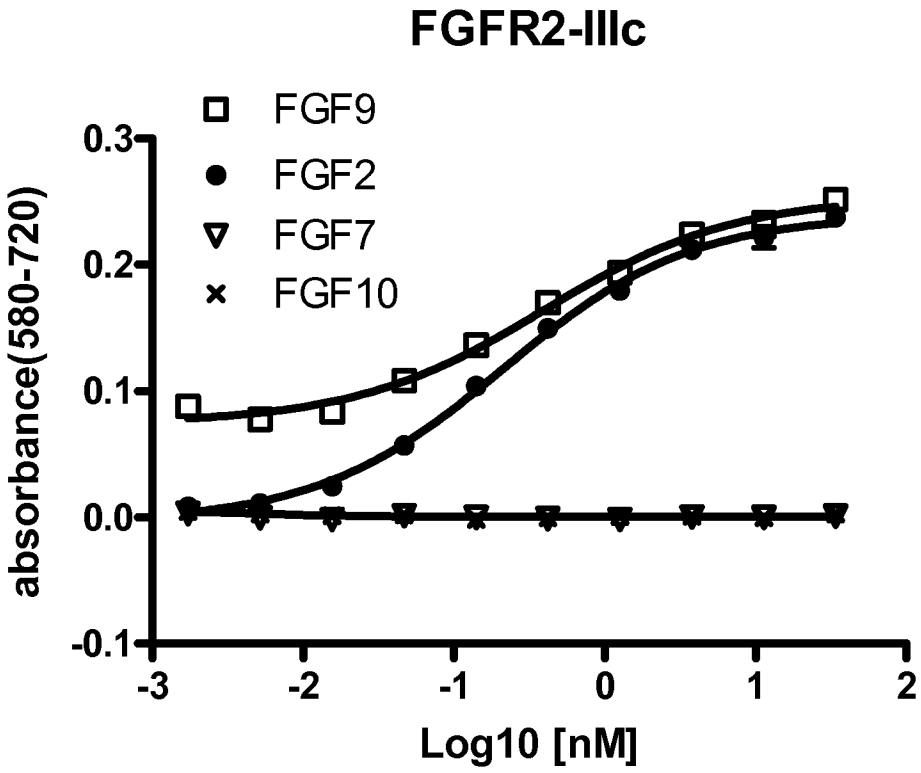

FGFR2-IIIc
FGF9
FGF2
FGF7
FGF10
absorbance(580-720)
0.3
0.2
0.1
0.0
-0.1
-3
-2
-1
0
1
2
Log10 [nM]

FIG. 4

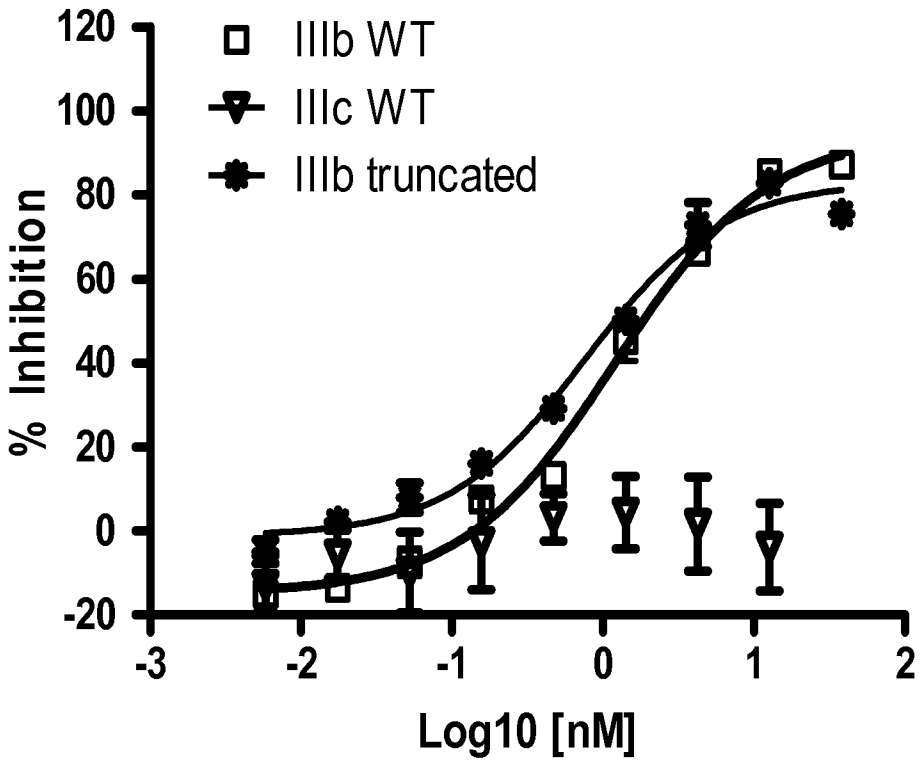

IIIb WT
IIIc WT
IIIb truncated
% Inhibition
120
100
80
60
40
20
0
-20
-3
-2
-1
0
1
2
Log10 [nM]

FIG. 5

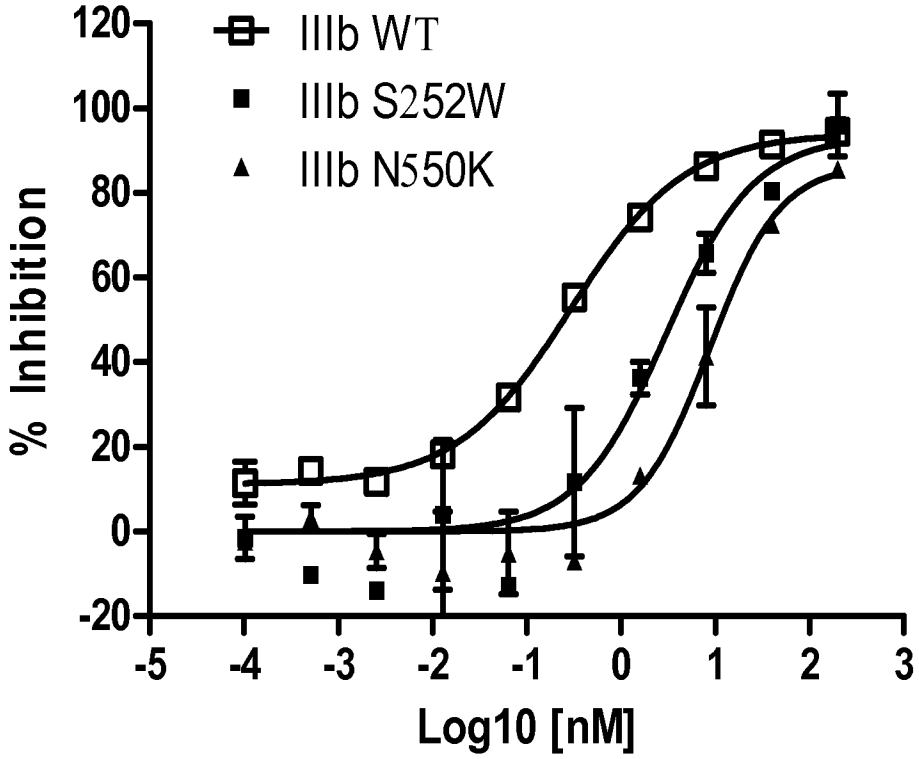

IIIb WT
IIIb S252W
IIIb N550K
% Inhibition
120
100
80
60
40
20
0
-20
-5
-4
-3
-2
-1
0
1
2
3
Log10 [nM]

FIG. 6

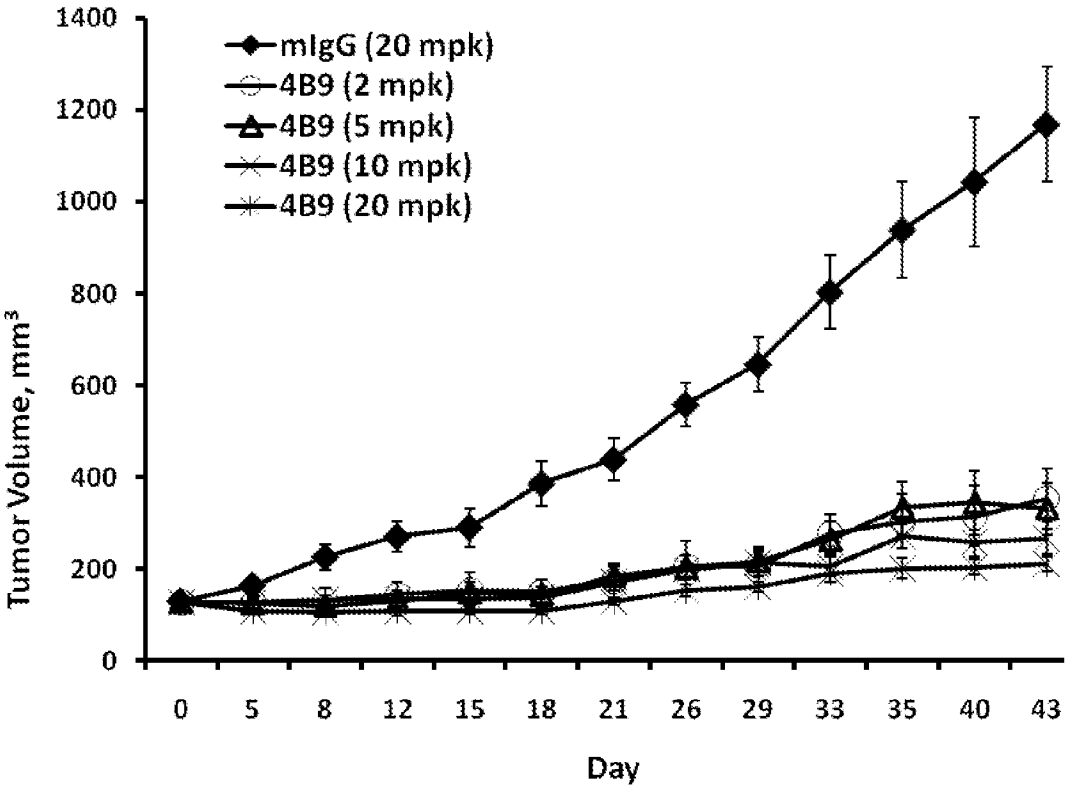
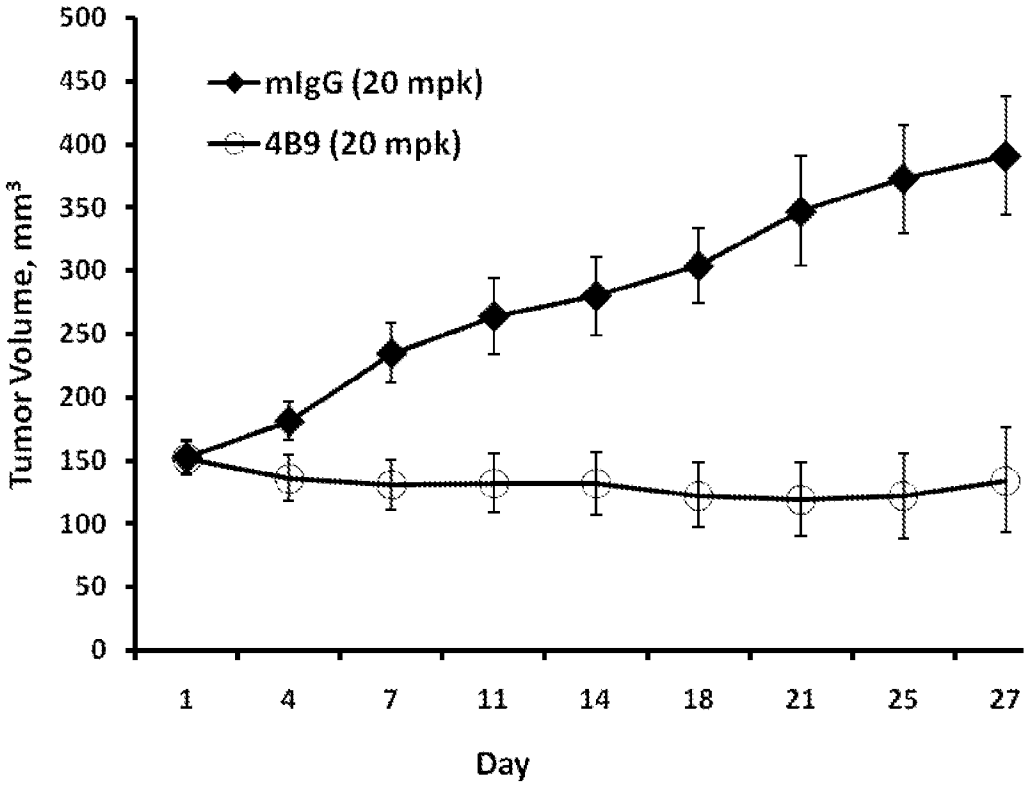

1400
1200
1000
800
600
400
200
0
Tumor Volume, mm³
mIgG (20 mpk)
4B9 (2 mpk)
4B9 (5 mpk)
4B9 (10 mpk)
4B9 (20 mpk)
0
5
8
12
15
18
21
26
29
33
35
40
43
Day

FIG. 7


500
450
400
350
300
250
200
150
100
50
0
Tumor Volume, mm³
mIgG (20 mpk)
4B9 (20 mpk)
1
4
7
11
14
18
21
25
27
Day

Complete Heavy Chain Variable Region Amino Acid Alignments

Antibody		CDR1	CDR2	CDR3	
4B9	Heavy	EVQLQQSGTVLARPGASVKMSCKTSGYTFT	SYWMH	WVKQRPGQGLEWIG	AIYPGNSDTDYSQKFKG	KATL	TAVTSATTAYMELSSLTNEDSAVYYCSK	FDY	WGQGTTLTVSS	(SEQ ID NO: 2)
Hu4B9-65	Heavy	QVQLVQSGAEVKKPGSSVKVSCKASGYTFT	SYWMH	WVRQAPGQGLEWMG	AIYPGNSDTDYSQKFKG	RVTI	TADESTSTAYMELSSLRSEDTAVYYCSK	FDY	WGQGTLVTVSS	(SEQ ID NO: 35)
Hu4B9-82, -83	Heavy	QVQLVQSGAEVKKPGSSVKVSCKASGYTFS	SYWMH	WVRQAPGQGLEWMG	AIYPGNSDTDYSQKFQG	RVTI	TADESTSTAYMELSSLRSEDTAVYYCSK	FDY	WGQGTLVTVSS	(SEQ ID NO: 37)

FIG. 8

Heavy Chain CDR Amino Acid Alignments

Antibody	CDR1	CDR2	CDR3
4B9 Heavy	SYWMH (SEQ ID NO: 5)	AIYPGNSDTDYSQKFKG (SEQ ID NO: 6)	FDY
Hu4B9-65 Heavy	SYWMH (SEQ ID NO: 5)	AIYPGNSDTDYSQKFKG (SEQ ID NO: 6)	FDY
Hu4B9-82, -83 Heavy	SYWMH (SEQ ID NO: 5)	AIYPGNSDTDYSQKFQG (SEQ ID NO: 38)	FDY

FIG. 9

Complete Light (Kappa) Chain Variable Region Amino Acid Alignments

Antibody		CDR1	CDR2
4B9 Kappa	QIVLTQSPALMSASPGEKVTMTC	SASSSVNYMY	WYQQKPRSSPKPWIY	LTSNLAS	GVPARFSGRGSGTSY
Hu4B9-65 Kappa	EIVLTQSPATLSLSPGERATLSC	RASSSVNYMY	WYQQKPGQAPRPWIY	LTSNRAT	GVPARFSGSGSGTDY
Hu4B9-82 Kappa	EIVLTQSPATLSLSPGERATLSC	RASSSVNYMY	WYQQKPGQAPRPWIY	LTSNRAT	GIPARFSGSGSGTDY
Hu4B9-83 Kappa	EIVLTQSPATLSLSPGERATLSC	RASSSVNYMY	WYQQKPGQAPRPWIY	LTSNRAT	GIPARFSGSGSGTDF

	CDR3	
4B9 Kappa	SLTISSMEAEDAATYYC	QQWSSNPYT	FGGGTKLEIK	(SEQ ID NO: 4)
Hu4B9-65 Kappa	TLTISSLEPEDFAVYYC	QQWSSNPYT	FGQGTKLEIK	(SEQ ID NO: 40)
Hu4B9-82 Kappa	TLTISSLEPEDFAVYYC	QQWSSNPYT	FGQGTKLEIK	(SEQ ID NO: 44)
Hu4B9-83 Kappa	TLTISSLEPEDFAVYYC	QQWSSNPYT	FGQGTKLEIK	(SEQ ID NO: 46)

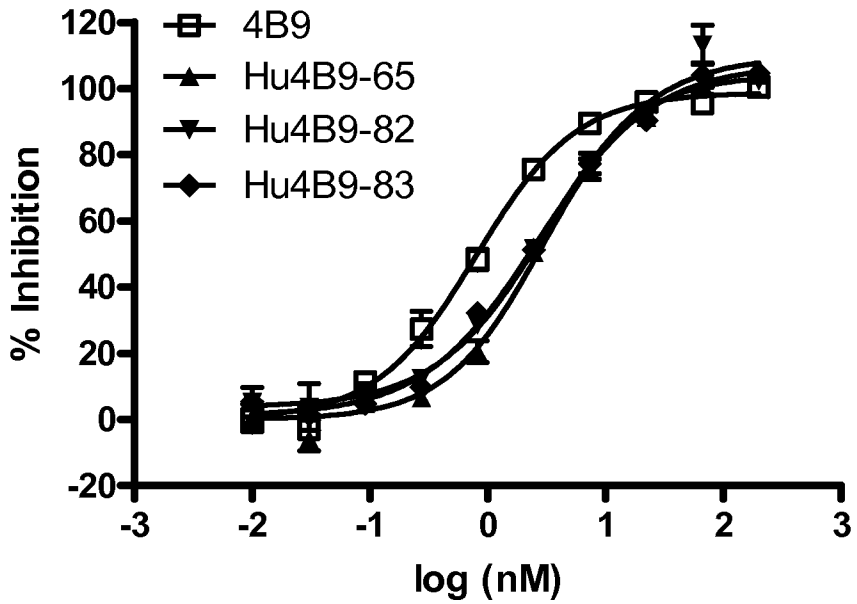
FIG. 10

Light (Kappa) Chain CDR Amino Acid Alignments

Antibody	CDR1	CDR2	CDR3
4B9 Kappa	SASSSVNYMY (SEQ ID NO: 12)	LTSNLAS (SEQ ID NO: 13)	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-65 Kappa	RASSSVNYMY (SEQ ID NO: 41)	LTSNRAT (SEQ ID NO: 42)	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-82 Kappa	RASSSVNYMY (SEQ ID NO: 41)	LTSNRAT (SEQ ID NO: 42)	QQWSSNPYT (SEQ ID NO: 14)
Hu4B9-83 Kappa	RASSSVNYMY (SEQ ID NO: 41)	LTSNRAT (SEQ ID NO: 42)	QQWSSNPYT (SEQ ID NO: 14)

FIG. 11

FIG. 12


4B9
Hu4B9-65
Hu4B9-82
Hu4B9-83
% Inhibition
120
100
80
60
40
20
0
-20
-3
-2
-1
0
1
2
3
log (nM)